Supplementary Information

Novel organophosphorus aminopyrimidines as unique structural DNA-targeting membrane active inhibitors towards drug-resistant methicillin-resistant Staphylococcus aureus

Di Li, Rammohan R. Yadav Bheemanaboina,# Narsaiah Battini,# Vijai Kumar Reddy Tangadanchu,§ Xian-Fu Fang and Cheng-He Zhou*

Institute of Bioorganic & Medicinal Chemistry, Key Laboratory of Applied Chemistry of Chongqing Municipality, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China.

 # Postdoctoral fellows from CSIR-Indian Institute of Integrative Medicine (IIIM), India.

§ Postdoctoral fellow from CSIR-Indian Institute of Chemical Technology (IICT), India.

** Corresponding author: zhouch@swu.edu.cn; Tel.: +86-23-68254967; Fax: +86-23-68254967.*

1 Experimental Protocols

1.1 Biological assays

The target compounds **2–8** were evaluated for their antimicrobial activities according to Clinical and Laboratory Standards Institute (CLSI) against eleven bacteria strains including Gram-positive bacteria (*Methicillin-Resistant Staphylococcus aureus*, *Staphylococcus aureus*, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis*) and Gram-negative bacteria (*Klebsiella pneumonia*, *Escherichia coli*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumanii*) that drug-resistant strains without ATCC number were isolated from infected patients, and fungi strains containing *Candida albicans*, *Candida albicans* ATCC 90023, *Candida tropicals*, *Aspergillus fumigatus*, *Candida parapsilosis* ATCC 22019. The bacterial and fungi suspension was adjusted with sterile saline to a concentration of 1×10^5 CFU which was measured by nephelometer. These synthesized compounds were dissolved in DMSO to prepare the stock solutions, and the tested compounds and reference drugs (chloromycin, norfloxacin and fluconazole) were prepared in Mueller-Hinton broth (Guangdong Huaikai Microbial Sci. & Tech Co., Ltd, Guangzhou, China) to give the required concentrations. These dilutions were inoculated and incubated at 37 °C for 24 h.

1.2 Resistance study

According to the previously reported method, the ative molecule **2c** was selected to explore the development of microbial resistance. Standard strain of resistant MRSA was exposed towards increasing concentrations of compound **2c** from sub-MIC (0.5 × MIC) for sustained passages, and then we determined the new MIC values of compound **2c** for each passage of MRSA. The initial MIC value of compound **2c** and norfloxacin was determined against MRSA as mentioned above in antimicrobial assay. For the next MIC experiment, the bacteria dilution was prepared from sub-MIC concentration (0.5 × MIC) of this compound. Twelve hours later, bacteria dilution was prepared once more by using the bacterial suspension from sub-MIC concentration of the compound (0.5 × MIC) and studied for the next MIC experiment.

1.3 Bactericidal kinetic assay

The rate of the highly active compound killed MRSA strain was evaluated by performing time-kill kinetics. MRSA strain was grown at 37 °C for 6 h, which was suitable growth medium, and diluted in respective media. Compound **2c** was added to the bacterial solution (MRSA of approximately 1.8×10^5 CFU/mL) at concentrations of MIC and 6 × MIC in a 96-well plate, and then the plate was then incubated at 37 °C. At different time intervals (0, 30, 60, 90, 120, 240, 360 and 420 min), this solution (20 μL) was taken out with and successively diluted (10-fold serial dilution) in 0.9% saline, and then this dilution was plated on respective agar plates and incubated at 37 °C for 24 h.

1.4 Cytotoxicity

The stock solutions of compound **2c** was prepared in medium and serially diluted in different concentrations. Normal mouse fibroblast L929 cells (Boster Biological Technology co.ltd, Tianjin, China) were seeded in a 96-well plate and cultured in a DMEM culture medium with 10% serum and 1% penicillin/streptomycin at 37 °C under 5% CO_2 atmosphere in an incubator for 24 h. The tested cells were treated with compound **2c** in triplicate at concentrations of 0, 4, 8, 16, 32, 64, 128, 256 and 512 μg/mL. Samples of different concentrations were then added into different wells and incubated with cells for another 48 h, 25 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-htetrazolium bromide (MTT) in phosphate-buffered saline (PBS) was added to each well and further incubated for 3–4 h. Later, the supernatant was removed and the cells were dissolved in 150 μL of DMSO. The optical density (OD) at 570 nm of each well was measured on a microplate reader (Bio-Rad 680). The absorbance values were measured by microplate reader at 490 nm.

1.5 Bacterial membrane permeabilization

MRSA strain was cultured at 37 °C. After 12 hours, this solution was centrifuged (3500 rpm, 5 min), washed and resuspended in 5 mM glucose and 5 mM HEPES buffer in 1:1 ratio in turn. Afterwards, 10 μL of compound **2c** (12 × MIC) was added to a cuvette which contained bacterial suspension and PI. Fluorescence was monitored at excitation wavelength of 535 nm (slit width of 10 nm) and emission wavelength of 617 nm (slit width of 5 nm). The uptake of PI was monitored by the increase in fluorescence for 10 min to measure inner membrane permeabilization.

1.6 Molecular docking

Autodock 4.2 was used to perform the docking work. The gird size was set to be 45 × 45 × 45 and the grid point spacing was set at default value 0.375 Å. The Lamarkian genetic algorithm (LGA) was applied for the conformational search.

1.7 Interactions of compound 2c with MRSA DNA

MRSA DNA was isolated from MRSA bacteria by a four-step process including lysis, digestion, precipitation and concentration, and its stock solution was prepared by dissolving DNA (3 mg) in doubly distilled water. The solution was allowed to stand overnight and store at 4 °C in the dark for a week. The concentration of DNA was determined by monitoring the ratio of the absorbance at 260 nm to that at 280 nm using TU-2450 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China) at room temperature. The solution gave a ratio of > 1.8 at A_{260}/A_{280}, which indicated that DNA was sufficiently free from protein. NR stock solution was prepared by dissolving its solid (Sigma Chemical Co.) in doubly distilled water and was kept in a cool and dark place. DNA was dissolved in Tris-hydrochloric acid (HCl) buffer solution (pH = 7.4), which was prepared by mixing and diluting Tris solution with HCl solution. Tris, HCl, ethanol were analytical purity.

2 General Procedure and Spectral Data for Some Representative Compounds

Pre-coated silica gel plates were employed to perform thin layer chromatography (TCL) analysis. Melting points of the synthesized compounds were recorded by using X-6 melting point apparatus (Beijing Focus Instrument CO., Ltd., China) and were uncorrected. Nuclear magnetic resonance (NMR) spectra were performed on a Bruker AV 600 spectrometer and the chemical shifts were estimated in parts per million (ppm), relative to tetramethylsilane (TMS) as an internal standard. The coupling constants (*J*) were expressed in hertz (Hz) and signals were described as singlet (s), doublet (d), triplet (t) and multiplet (m).

*Synthesis of diethyl ((2-butyl-4-chloro-1-ethyl-1H-imidazol-5-yl)(pyrimidin-2-ylamino)methyl)phosphonate (**2a**).* A solution of 2-butyl-4-chloro-1-ethyl-1*H*-imidazole-5-carbaldehyde (0.850 g, 3.959 mmol), diethyl phosphonate (0.656 g, 4.751 mmol) and 2-aminopyrimidine (0.377 g, 3.959 mmol) in toluene was refluxed at 115 °C under magnetic stirring for 5 h. After the reaction completed, the reactive mixture was concentrated by reduced pressure to provide the crude product, which was further purified by silica gel column chromatography (eluent, methanol/dichloromethane, 2/30, V/V) to produce 1.137 g of compound **2a** as yellow solid. Yield: 66.8%; mp: 124.9–125.6 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.21 (d, *J* = 4.8 Hz, 2H, pyrimidine-4,6-2*H*), 7.24 (s, 1H, pyrimidine-2-N*H*), 6.75 (t, *J* = 4.8 Hz, 1H, pyrimidine-5-*H*), 5.79 (dd, *J* = 25.2, 9.5 Hz, 1H, C*H*), 4.30 (dd, *J* = 14.8, 7.2 Hz, 1H, NC*H*CH_3), 4.07 (dt, *J* = 14.4, 7.1 Hz, 2H, OCH_2CH_3), 4.05–3.95 (m, 2H, OCH_2CH_3), 3.88 (dd, *J* = 16.4, 9.1 Hz, 1H, NC*H*CH_3), 2.58 (td, *J* = 7.4, 3.3 Hz, 2H, C$H_2CH_2CH_2CH_3$), 1.66–1.54 (m, 2H, $CH_2CH_2CH_2CH_3$), 1.34 (dq, *J* = 14.7, 7.4 Hz, 2H, $CH_2CH_2CH_2CH_3$), 1.25 (t, *J* = 6.9 Hz, 3H, NCH_2CH_3), 1.19 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.10 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 0.89 (t, *J* = 7.4 Hz, 3H, $CH_2CH_2CH_2CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.1, 164.1, 158.5, 147.4, 119.7, 112.6, 110.6, 63.2, 49.1, 44.5, 43.4, 40.6, 29.60, 26.0, 22.2, 16.6, 16.5, 14.2 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.5 ppm; HRMS calcd. for $C_{18}H_{29}ClN_5O_3P$ $[M+Na]^+$, 452.1594; found, 452.1596.

*Synthesis of diethyl ((2-butyl-4-chloro-1-propyl-1H-imidazol-5-yl)(pyrimidin-2-ylamino)methyl)phosphonate (**2b**).* The target compound **2b** (1.500 g) as yellow solid was prepared for 5 h according to general procedure described for **2a** starting from 2-butyl-4-chloro-1-propyl-1*H*-imidazole-5-carbaldehyde (1.203 g, 5.256 mmol), diethyl phosphonate (0.871 g, 6.312 mmol) and 2-aminopyrimidine (0.500 g, 5.256 mmol). Yield: 64.3%; mp: 113.5–114.6 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.37 (d, *J* = 4.7 Hz, 2H, pyrimidine-4,6-2*H*), 8.21 (d, *J* = 4.7 Hz, 1H, pyrimidin-2-N*H*), 6.74 (t, *J* = 4.7 Hz, 1H, pyrimidine-5-*H*), 5.75 (dd, *J* = 24.9, 9.3 Hz, 1H, C*H*), 4.15 (dd, *J* = 14.5, 8.9 Hz, 1H, NC*H*CH_2CH_3), 4.07 (dd, *J* = 15.0, 7.7 Hz, 2H, OCH_2CH_3), 3.99–3.95 (m, 1H, NC*H*CH_2CH_3), 3.89 (dd, *J* = 15.4, 9.2 Hz, 2H, OCH_2CH_3), 2.56 (d, *J* = 7.4 Hz, 2H, C$H_2CH_2CH_2CH_3$), 1.67–1.56 (m, 6H, $NCH_2CH_2CH_3$, $CH_2CH_2CH_2CH_3$), 1.34 (dd, *J* = 14.9, 7.4 Hz, 3H, $NCH_2CH_2CH_3$), 1.25 (dd, *J* = 12.3, 5.2 Hz, 3H, OCH_2CH_3), 1.19 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.10 (t, *J* = 7.0 Hz, 3H, $CH_2CH_2CH_2CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.4, 161.3, 158.5, 147.7, 119.9, 112.6, 110.6, 63.2, 45.9, 44.6, 43.5, 40.6, 29.7, 26.1, 23.9, 22.2, 16.7, 16.5, 14.2 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.4 ppm; HRMS calcd. for $C_{19}H_{31}ClN_5O_3P$ $[M+Na]^+$, 466.1751; found, 466.1750.

*Synthesis of diethyl ((2-butyl-4-chloro-1-pentyl-1H-imidazol-5-yl)(pyrimidin-2-ylamino)methyl)phosphonate (**2c**).* The target compound **2c** (1.072 g) as yellow solid was prepared for 6 h according to general procedure described for **2a** starting from 2-butyl-4-chloro-1-pentyl-1*H*-imidazole-5-carbaldehyde (1.350 g, 5.256 mmol), diethyl phosphonate (0.871 g, 6.312 mmol) and 2-aminopyrimidine (0.500 g, 5.256 mmol). Yield: 43.2%; mp: 109.8–110.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.38 (d, *J* = 4.6 Hz, 2H, pyrimidine-4,6-2*H*), 7.02 (s, 1H, pyrimidine-2-N*H*), 6.76 (t, *J* = 4.7 Hz, 1H, pyrimidine-5-*H*), 5.74 (dd, *J* = 24.8, 9.3 Hz, 1H, C*H*), 4.19–4.13 (m, 1H, NC*H*$(CH_2)_3CH_3$), 4.10–4.04 (m, 2H, OCH_2CH_3), 3.99 (dd, *J* = 17.0, 7.6 Hz, 1H, NC*H*$(CH_2)_3CH_3$), 3.95–3.85 (m, 2H, OCH_2CH_3), 2.57 (t, *J* = 7.5 Hz, 2H, C$H_2CH_2CH_2CH_3$), 1.70 (s, 1H, $CH_2CHCH_2CH_3$), 1.61 (dt, *J* = 15.0, 7.4 Hz, 3H, $CH_2CHCH_2CH_3$, $NCH_2CH_2CH_2CH_2CH_3$), 1.33 (dd, *J* = 15.9,

8.3 Hz, 6H, $CH_2CH_2CH_2CH_3$, $NCH_2CH_2CH_2CH_2CH_3$), 1.19 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.10 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 0.87 (dt, *J* = 13.6, 6.7 Hz, 6H, $CH_2CH_2CH_2CH_3$, $N(CH_2)_4CH_3$)) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.3, 161.3, 158.7, 147.5, 126.9, 119.8, 112.6, 63.2, 44.6, 44.4, 43.5, 40.6, 30.2, 29.7, 28.6, 26.1, 22.3, 22.2, 16.6, 16.5, 14.2 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 19.4 ppm; HRMS calcd. for $C_{21}H_{35}ClN_5O_3P$ $[M+H]^+$, 472.2244; found, 472.2240; $[M+Na]^+$, 494.2064; found, 494.2063.

*Synthesis of diethyl ((2-butyl-4-chloro-1-(prop-2-yn-1-yl)-1H-imidazol-5-yl)(pyrimidin-2-ylamino)methyl)phosphonate (**3**)*. The target compound **3** (1.071 g) as white solid was prepared for 7 h according to general procedure described for **2a** starting from 2-butyl-4-chloro-1-(prop-2-yn-1-yl)-1*H*-imidazole-5-carbaldehyde (1.000 g, 4.450 mmol), diethyl phosphonate (0.737 g, 5.340 mmol) and 2-aminopyrimidine (0.423 g, 4.450 mmol). Yield: 54.7%; mp: 113.5–114.6 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.57 (d, *J* = 4.8 Hz, 2H, pyrimidine-4,6-2*H*), 7.09 (s, 1H, pyrimidine-2-N*H*), 7.00 (t, *J* = 4.8 Hz, 1H, pyrimidine-5-*H*), 6.52 (d, *J* = 18.7 Hz, 1H, C*H*), 4.09–4.00 (m, 2H, OCH_2CH_3), 3.99–3.94 (m, 2H, OCH_2CH_3), 2.69 (t, *J* = 7.5 Hz, 2H, $CH_2(CH_2)_2CH_3$), 2.31 (s, 1H, ≡C*H*), 2.31 (d, *J* = 0.7 Hz, 2H, CH_2), 1.62–1.54 (m, 2H, $CH_2CH_2CH_2CH_3$), 1.33 (dq, *J* = 14.7, 7.4 Hz, 2H, $CH_2CH_2CH_2CH_3$), 1.17 (t, *J* = 7.0, 1.4 Hz, 3H, OCH_2CH_3), 1.17 (t, *J* = 7.0, 1.4 Hz, 3H, OCH_2CH_3), 0.89 (t, *J* = 7.4 Hz, 3H, $CH_2(CH_2)_2CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.8, 163.8, 163.4, 148.7, 131.7, 127.4, 120.6, 119.1, 116.2, 67.8, 53.3, 52.2, 34.6, 30.5, 26.8, 23.4, 21.4, 21.4, 18.8 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 22.2 ppm; HRMS calcd. for $C_{19}H_{27}ClN_5O_3P$ $[M+H]^+$, 440.1618; found, 440.1613.

*Synthesis of diethyl ((4-nitrophenyl)(pyrimidin-2-ylamino)methyl)phosphonate (**4a**)*. The target compound **4a** (1.122 g) as white solid was prepared for 4 h according to general procedure described for **2a** starting from 4-nitrobenzaldehyde (0.796 g, 5.256 mmol), diethyl phosphonate (0.871 g, 6.312 mmol) and 2-aminopyrimidine (0.500 g, 5.256 mmol). Yield: 58.3%; mp: 135.5–136.1 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.34 (d, *J* = 4.5 Hz, 2H, pyrimidine-4,6-2*H*), 8.22 (d, *J* = 8.6 Hz, 2H, 4-NO_2Ph-3,5-2*H*), 8.19 (dd, *J* = 9.9, 2.6 Hz, 1H, pyrimidine-2-N*H*), 7.85 (d, *J* = 7.3 Hz, 2H, 4-NO_2Ph-2,6-2*H*), 6.69 (t, *J* = 4.7 Hz, 1H, pyrimidine-5-*H*), 5.91 (dd, *J* = 23.8, 10.0 Hz, 1H, C*H*), 4.02–3.98 (m, 2H, OCH_2CH_3), 3.93 (dt, *J* = 17.2, 8.4 Hz, 2H, OCH_2CH_3), 1.14–1.09 (m, 6H, $2OCH_2CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.8, 161.8, 158.6, 147.4, 145.0, 129.9, 129.9, 123.6, 123.6, 112.1, 63.3, 52.8, 51.8, 16.6, 16.5 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 20.5 ppm; HRMS calcd. for $C_{15}H_{19}N_4O_5P$ $[M+H]^+$, 367.1171; found, 367.1181; $[M+Na]^+$, 389.0991; found, 389.0992.

*Synthesis of diethyl ((2,4-dichlorophenyl)(pyrimidin-2-ylamino)methyl)phosphonate (**4b**)*. The target compound **4b** (1.267 g) as colourless liquid was prepared for 3 h according to general procedure described for **2a** starting from 2,4-dichlorobenzaldehyde (0.920 g, 5.256 mmol), diethyl phosphonate (0.871 g, 6.312 mmol) and 2-aminopyrimidine (0.500 g, 5.256 mmol). Yield: 61.8%; mp: < 25 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.35 (d, *J* = 4.7 Hz, 2H, pyrimidine-4,6-2*H*), 8.07 (d, *J* = 9.6 Hz, 1H, 2,4-Cl_2Ph-3-*H*), 7.93 (d, *J* = 6.8 Hz, 1H, 2,4-Cl_2Ph-5-*H*), 7.66 (d, *J* = 8.4 Hz, 1H, 2,4-Cl_2Ph-6-*H*), 7.49 (d, *J* = 6.9 Hz, 1H, pyrimidine-2-N*H*), 6.69 (t, *J* = 4.7 Hz, 1H, pyrimidine-5-*H*), 6.26 (dd, *J* = 22.4, 10.0 Hz, 1H, C*H*), 4.06–4.02 (m, 2H, OCH_2CH_3), 3.97–3.90 (m, 2H, OCH_2CH_3), 1.20–1.09 (m, 6H, $2OCH_2CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.7, 161.6, 158.6, 134.5, 133.5, 132.1, 131.7, 128.9, 128.7, 112.1, 62.7, 49.1, 48.9, 16.64, 16.5 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 20.6 ppm; HRMS calcd. for $C_{15}H_{18}Cl_2N_3O_3P$ $[M+Na]^+$, 412.0360; found, 412.0361.

*Synthesis of diethyl ((2-chlorophenyl)(pyrimidin-2-ylamino)methyl)phosphonate (**4c**)*. The target compound **4c** (1.030 g) as yellow liquid was prepared for 5 h according to general procedure described for **2a** starting from 2-chlorobenzaldehyde (0.741 g, 5.256 mmol), diethyl phosphonate (0.871 g, 6.312 mmol) and 2-aminopyrimidine (0.500 g, 5.256 mmol). Yield: 55.1%; mp: < 25 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.35 (d, *J* = 4.7 Hz, 2H, pyrimidine-4,6-2*H*), 7.98 (d, *J* = 9.8 Hz, 1H, pyrimidine-2-N*H*), 7.91 (d, *J* = 7.6 Hz, 1H, 2-ClPh-3-*H*), 7.48–7.41 (m, 2H, 2-ClPh-4,5-2*H*), 7.32 (d, J = 7.7 Hz, 1H, 2-ClPh-6-*H*), 6.68 (t, *J* = 4.6 Hz, 1H, pyrimidine-5-*H*), 6.32 (dd, *J* = 22.3, 10.1 Hz, 1H, C*H*), 4.03 (d, *J* = 7.1 Hz, 2H, OCH_2CH_3), 3.95–3.88 (m, 2H, OCH_2CH_3), 1.13 (dt, *J* = 14.4, 7.0 Hz, 6H, $2OCH_2CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.8, 161.7, 158.6, 136.8, 133.6, 130.8, 130.4, 129.4, 127.6, 112.0, 63.0, 49.2, 48.2, 16.6, 16.6 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 21.2 ppm; HRMS calcd. for $C_{15}H_{19}ClN_3O_3P$ $[M+H]^+$, 356.0931; found, 356.0932; $[M+Na]^+$, 378.0750; found, 378.0752.

*Synthesis of diethyl ((4-chlorophenyl)(pyrimidin-2-ylamino)methyl)phosphonate (**4d**)*. The target compound **4d** (1.135 g) as white solid was prepared for 4 h according to general procedure described for **2a** starting from 4-chlorobenzaldehyde (0.741 g, 5.256 mmol), diethyl phosphonate (0.871 g, 6.312 mmol) and 2-aminopyrimidine (0.500 g, 5.256 mmol). Yield: 60.7%; mp: 102.8–103.4 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.33 (d, *J* = 4.7 Hz, 2H, pyrimidine-4,6-2*H*), 8.01 (d, *J* = 9.9 Hz, 1H, pyrimidine-2-N*H*), 7.58 (d, *J* = 7.0 Hz, 2H, 4-ClPh-3,5-2*H*), 7.41 (d, *J* = 8.3 Hz, 2H, 4-ClPh-2,6-2*H*), 6.67 (t, *J* = 4.7 Hz, 1H, pyrimidine-5-*H*), 5.74 (dd, *J* = 22.8, 10.2 Hz, 1H, C*H*), 3.99–3.94 (m, 2H, OCH_2CH_3), 3.94–3.81 (m, 2H, OCH_2CH_3), 1.11 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.08 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.9, 161.8, 158.5, 136.0, 132.6, 130.6, 130.6, 128.5, 128.5, 111.8, 63.0, 52.2, 51.2, 16.7, 16.6 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 21.5 ppm; HRMS calcd. for $C_{15}H_{19}ClN_3O_3P$ $[M+H]^+$, 356.0931; found, 356.0930; $[M+Na]^+$, 378.0750; found, 378.0751.

*Synthesis of diethyl ((4-fluorophenyl)(pyrimidin-2-ylamino)methyl)phosphonate (**4e**)*. The target compound **4e** (1.059 g) as white solid was prepared for 5 h according to general procedure described for **2a** starting from 4-fluorobenzaldehyde (0.653 g, 5.256 mmol), diethyl phosphonate (0.871 g, 6.312 mmol) and 2-aminopyrimidine (0.500 g, 5.256 mmol). Yield: 59.4%; mp: 109.5–110.0 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.33 (d, *J* = 4.7 Hz, 2H, pyrimidine-4,6-2*H*), 7.98 (d, *J* = 10.1 Hz, 1H, pyrimidine-2-N*H*), 7.63–7.57 (m, 2H, 4-FPh-3,5-2*H*), 7.17 (t, *J* = 8.8 Hz, 2H, 4-FPh-2,6-2*H*), 6.66 (t, *J* = 4.7 Hz, 1H, pyrimidine-5-*H*), 5.76 (dd, *J* = 22.5, 10.2 Hz, 1H, C*H*), 3.98–3.94 (m, 2H, OCH_2CH_3), 3.93–3.81 (m, 2H, OCH_2CH_3), 1.11 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 1.07 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.9, 161.9, 158.5, 133.1, 130.8, 130.7, 115.4, 115.2, 111.8, 111.8, 62.8, 52.0, 51.0, 16.6, 16.5 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 21.8 ppm; HRMS calcd. for $C_{15}H_{19}FN_3O_3P$ $[M+H]^+$, 340.1226; found, 362.1218; $[M+Na]^+$, 362.1046; found, 362.1044.

*Synthesis of diethyl ((pyrimidin-2-ylamino)(p-tolyl)methyl)phosphonate (**4f**)*. The target compound **4f** (1.038 g) as white solid was prepared for 4 h according to general procedure described for **2a** starting from 4-methylbenzaldehyde (0.633 g, 5.256 mmol), diethyl phosphonate (0.871 g, 6.312 mmol) and 2-aminopyrimidine (0.500 g, 5.256 mmol). Yield: 58.9%; mp: 102.4–102.8 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.32 (d, J = 4.7 Hz, 2H, pyrimidine-4,6-2H), 7.85 (d, J = 10.2 Hz, 1H, pyrimidine-2-NH), 7.42 (d, J = 6.6 Hz, 2H, 4-CH_3Ph-2,6-2H), 7.14 (d, J = 7.9 Hz, 2H, 4-CH_3Ph-3,5-2H), 6.65 (t, J = 4.7 Hz, 1H, pyrimidine-5-H), 5.69 (dd, J = 22.4, 10.2 Hz, 1H, CH), 3.96–3.92 (m, 2H, OCH_2CH$_3$), 3.90–3.77 (m, 2H, OCH_2CH$_3$), 2.27 (s, 3H, CH_3), 1.11 (t, J = 7.1 Hz, 3H, OCH$_2$CH_3), 1.07 (t, J = 7.0 Hz, 3H, OCH$_2$CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.9, 161.9, 158.5, 137.1, 133.8, 129.1, 129.1, 128.7, 128.7, 111.7, 62.7, 52.4, 51.4, 21.1, 16.6, 16.5 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 22.2 ppm; HRMS calcd. for $C_{16}H_{22}N_3O_3P$ [M+Na]$^+$, 358.1296; found, 358.1298.

*Synthesis of diethyl ((4-methoxyphenyl)(pyrimidin-2-ylamino)methyl)phosphonate (**4g**)*. The target compound **4g** (0.982 g) as white solid was prepared for 3 h according to general procedure described for **2a** starting from 4-methoxybenzaldehyde (0.716 g, 5.256 mmol), diethyl phosphonate (0.871 g, 6.312 mmol) and 2-aminopyrimidine (0.500 g, 5.256 mmol). Yield: 53.2%; mp: 102.3–102.7 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.32 (d, J = 4.7 Hz, 2H, pyrimidine-4,6-2H), 7.85 (d, J = 10.1 Hz, 1H, pyrimidine-2-NH), 7.47 (d, J = 10.3 Hz, 2H, 4-OCH$_3$Ph-2,6-2H), 6.89 (d, J = 8.6 Hz, 2H, 4-OCH$_3$Ph-3,5-2H), 6.65 (t, J = 4.7 Hz, 1H, pyrimidine-5-H), 5.67 (dd, J = 22.1, 10.2 Hz, 1H, CH), 3.98–3.92 (m, 2H, OCH_2CH$_3$), 3.84 (dd, J = 28.1, 19.6 Hz, 2H, OCH_2CH$_3$), 3.73 (s, 3H, OCH_3), 1.11 (t, J = 7.0 Hz, 3H, OCH$_2$CH_3), 1.06 (t, J = 7.0 Hz, 3H, OCH$_2$CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.9, 161.9, 159.2, 130.0, 129.9 128.7, 114.0, 114.0, 111.6, 111.6, 62.6, 55.5, 52.0, 51.0, 16.6, 16.5; ^{31}P NMR (243 MHz, DMSO-d_6) δ 22.3 ppm; HRMS calcd. for $C_{16}H_{22}N_3O_4P$ [M+Na]$^+$, 374.1246; found, 374.1244.

*Synthesis of ((4-nitrophenyl)(pyrimidin-2-ylamino)methyl)phosphonic acid (**5a**)*. Compound **4a** (0.050 g, 0.137 mmol) in concentrated hydrochloric acid (1 mL) was refluxed for 12 h. On cooling to room temperature, the reaction mixture was diluted with water and washed with ethyl acetate. The water layer was concentrated and co-evaporated with ethanol. The resulting precipitate was suspended with water/methanol (1/1) and collected by filtration to give 0.027 g of compound **5a** as white solid. Yield: 62.2%; mp: > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.30 (d, J = 3.0 Hz, 2H, pyrimidine-4,6-2H), 8.18 (d, J = 8.6 Hz, 2H, 4-NO_2Ph-3,5-2H), 7.74 (d, J = 7.1 Hz, 2H, 4-NO_2Ph-2,6-2H), 7.43–7.36 (m, 1H, pyrimidine-2-NH), 6.66 (t, J = 4.8 Hz, 1H, pyrimidine-5-H), 5.49 (dd, J = 23.4, 9.1 Hz, 1H, CH) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.9, 161.8, 158.6, 147.3, 147.0, 147.0, 147.0, 129.5, 123.4, 111.9, 54.3 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 15.2 ppm; HRMS calcd. for $C_{11}H_{11}N_4O_5P$ [M+H]$^+$, 311.0545; found, 311.0553; [M+Na]$^+$, 333.0365; found, 333.0381.

*Synthesis of ((4-fluorophenyl)(pyrimidin-2-ylamino)methyl)phosphonic acid (**5b**)*. The target compound **5b** (0.051 g) as white solid was prepared for 12 h according to general procedure described for **5a** starting from compound **4e** (0.100 g, 0.295 mmol). Yield: 63.0%; mp: > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.29 (d, J = 4.7 Hz, 2H, pyrimidine-4,6-2H), 7.54–7.45 (m, 2H, 4-FPh-3,5-2H), 7.31–7.23 (m, 1H, pyrimidine-2-NH), 7.12 (t, J = 8.8 Hz, 2H, 4-FPh-2,6-2H), 6.63 (t, J = 4.8 Hz, 1H, pyrimidine-5-H), 5.39 (dd, J = 22.4, 9.5 Hz, 1H, CH) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.0, 161.9, 158.5, 135.1, 130.3, 130.3, 115.0, 114.9, 111.6, 111.6, 53.3 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 17.3 ppm; HRMS calcd. for $C_{11}H_{11}FN_3O_3P$ [M+H]$^+$, 284.0600; found, 284.0601; [M+Na]$^+$, 306.0420; found, 306.0422.

*Synthesis of ((pyrimidin-2-ylamino)(p-tolyl)methyl)phosphonic acid (**5c**)*. The target compound **5c** (0.045 g) as white solid was prepared for 12 h according to general procedure described for **5a** starting from compound **4f** (0.100 g, 0.298 mmol). Yield: 53.2%; mp: > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.32 (d, J = 4.8 Hz, 2H, pyrimidine-4,6-2H), 7.33 (d, J = 6.6 Hz, 2H, 4-CH_3Ph-3,5-2H), 7.09 (d, J = 7.9 Hz, 2H, 4-CH_3Ph-2,6-2H), 6.66 (t, J = 4.8 Hz, 1H, pyrimidine-5-H), 5.35 (dd, J = 23.4, 9.1 Hz, 1H, CH) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.8, 161.8, 158.4, 136.3, 135.8, 128.8, 128.8, 128.4, 128.3, 111.4, 53.6, 21.1 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 17.0 ppm; HRMS calcd. for $C_{12}H_{14}N_3O_3P$ [M+H]$^+$, 280.0851; found, 280.0849; [M+Na]$^+$, 302.0670; found, 302.0673.

*Synthesis of diethyl ((1H-indol-3-yl)(pyrimidin-2-ylamino)methyl)phosphonate (**6a**)*. The target compound **6a** (0.739 g) as white solid was prepared for 6 h according to general procedure described for **2a** starting from 1H-indole-3-carbaldehyde (0.490 g, 3.378 mmol), diethyl phosphonate (0.563 g, 4.054 mmol) and 2-aminopyrimidine (0.321 g, 3.378 mmol). Yield: 60.7%; mp: 156.5–157.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.09 (s, 1H, benzazole-NH), 8.35 (s, 2H, pyrimidine-4,6-2H), 7.62 (dd, J = 16.9, 9.1 Hz, 3H, benzazole-2,4-2H, pyrimidine-2-NH), 7.37 (d, J = 8.1 Hz, 1H, benzazole-7-H), 7.09 (t, J = 7.5 Hz, 1H, benzazole-6-H), 7.00 (t, J = 7.5 Hz, 1H, benzazole-5-H), 6.65 (t, J = 4.7 Hz, 1H, pyrimidine-5-H), 6.09 (dd, J = 20.6, 10.1 Hz, 1H, CH), 4.05–3.96 (m, 2H, OCH_2CH$_3$), 3.94–3.79 (m, 2H, OCH_2CH$_3$), 1.11 (t, J = 7.0 Hz, 3H, OCH$_2$CH_3), 1.03 (t, J = 7.0 Hz, 3H, OCH$_2$CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.9, 161.9, 158.5, 136.2, 126.9, 125.5, 121.7, 119.2, 119.2, 111.9, 111.3, 109.9, 62.5, 44.9, 43.8, 16.7, 16.6 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 23.1 ppm; HRMS calcd. for $C_{17}H_{21}N_4O_3P$ [M+Na]$^+$, 383.1249; found, 383.1243.

*Synthesis of diethyl ((1-ethyl-1H-indol-3-yl)(pyrimidin-2-ylamino)methyl)phosphonate (**6b**)*. The target compound **6b** (0.566 g) as white solid was prepared for 5 h according to general procedure described for **2a** starting from 1-ethyl-1H-indole-3-carbaldehyde (0.500 g, 2.887 mmol), diethyl phosphonate (0.478 g, 3.464 mmol) and 2-aminopyrimidine (0.275 g, 2.887 mmol). Yield: 50.5%; mp: 131.1–132.4 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.34 (d, J = 2.5 Hz, 2H, pyrimidine-4,6-2H), 7.62 (dd, J = 12.1, 4.9 Hz, 2H, benzazole-2,4-2H), 7.58 (d, J = 9.9 Hz, 1H, pyrimidine-2-NH), 7.44 (d, J = 8.2 Hz, 1H, benzazole-7-H), 7.13 (t, J = 7.8 Hz, 1H, benzazole-6-H), 7.02 (t, J = 7.5 Hz, 1H, benzazole-5-H), 6.64 (t, J = 4.7 Hz, 1H, pyrimidine-5-H), 6.07 (dd, J = 20.7, 10.1 Hz, 1H, CH), 4.18 (q, J = 7.2 Hz, 2H, NCH_2CH$_3$), 4.04–3.94 (m, 2H, OCH_2CH$_3$), 3.94–3.78 (m, 2H, OCH_2CH$_3$), 1.34 (t, J = 7.2 Hz, 3H, NCH$_2$CH_3), 1.10 (t, J = 7.0 Hz, 3H, OCH$_2$CH_3), 1.01 (t, J = 7.0 Hz, 3H, OCH$_2$CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.9, 161.9, 158.5 135.8, 127.9, 127.3, 121.7, 119.7, 119.3, 111.4, 110.2, 109.3, 62.5, 49.1, 44.8, 43.8, 40.8, 16.6, 15.8 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 22.9 ppm; HRMS calcd. for $C_{19}H_{25}N_4O_3P$ [M+H]$^+$,

389.1743; found, 389.1735.

*Synthesis of diethyl ((2-oxo-2H-chromen-6-yl)(pyrimidin-2-ylamino)methyl)phosphonate (**7**)*. The target compound **7** (0.157 g) as white solid was prepared for 5 h according to general procedure described for **2a** starting from 2-oxo-2*H*-chromene-6-carbaldehyde (0.200 g, 1.148 mmol), diethyl phosphonate (0.190 g, 1.378 mmol) and 2-aminopyrimidine (0.109 g, 1.148 mmol). Yield: 35.1%; mp: 102.5–103.1 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.36 (d, *J* = 4.7 Hz, 2H, pyrimidine-4,6-2*H*), 8.05 (d, *J* = 9.6 Hz, 2H, coumarin-4,7-2*H*), 7.90 (s, 1H, coumarin-5-*H*), 7.85 (d, *J* = 8.6 Hz, 1H, pyrimidine-2-N*H*), 7.41 (d, *J* = 8.6 Hz, 1H, coumarin-8-*H*), 6.69 (t, *J* = 4.7 Hz, 1H, pyrimidine-5-*H*), 6.50 (d, *J* = 9.6 Hz, 1H, coumarin-3-*H*), 5.84 (dd, *J* = 22.5, 10.1 Hz, 1H, C*H*), 4.01 (dd, *J* = 14.7, 7.5 Hz, 2H, OCH_2CH$_3$), 3.93 (dd, *J* = 37.2, 8.7 Hz, 2H, OCH_2CH$_3$), 1.14 (t, *J* = 7.0 Hz, 3H, OCH$_2$CH_3), 1.10 (t, *J* = 7.0 Hz, 3H, OCH$_2$CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.8, 160.3, 158.6, 153.4, 144.5, 133.4, 132.3, 128.4, 118.8, 117.0, 116.6, 111.9, 63.0, 62.8, 52.1, 51.1, 16.6, 16.6 ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 21.5 ppm; HRMS calcd. for $C_{18}H_{20}N_3O_5P$ $[M+H]^+$, 390.1219; found, 390.1212; $[M+Na]^+$, 412.1038; found, 412.1037.

*Synthesis of diethyl ((9-ethyl-6-formyl-9H-carbazol-3-yl)(pyrimidin-2-ylamino)methyl)phosphonate (**8**)*. The target compound **8** (0.153 g) as yellow solid was prepared for 5 h according to general procedure described for **2a** starting from 9-ethyl-9*H*-carbazole-3,6-dicarbaldehyde (0.264 g, 1.051 mmol), diethyl phosphonate (0.174 g, 1.261 mmol) and 2-aminopyrimidine (0.100 g, 1.051 mmol). Yield: 31.2%; mp: 156.0–157.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.07 (s, 1H, carbazole-6-C*H*O), 8.71 (s, 1H, carbazole-5-*H*), 8.51 (s, 1H, carbazole-4-*H*), 8.34 (d, *J* = 4.7 Hz, 2H, pyrimidine-4,6-2*H*), 8.00 (d, *J* = 7.3 Hz, 1H, carbazole-8-*H*), 7.95 (d, *J* = 10.3 Hz, 1H, pyrimidine-2-N*H*), 7.79 (d, *J* = 8.5 Hz, 1H, carbazole-1-*H*), 7.76 (d, *J* = 8.5 Hz, 1H, carbazole-7-*H*), 7.69 (d, *J* = 8.5 Hz, 1H, carbazole-2-*H*), 6.66 (t, *J* = 4.7 Hz, 1H, pyrimidine-5-*H*), 5.93 (dd, *J* = 21.9, 10.2 Hz, 1H, C*H*), 4.50 (q, *J* = 7.1 Hz, 2H, NCH_2CH$_3$), 4.04–3.97 (m, 2H, OCH_2CH$_3$), 3.88 (dd, *J* = 44.8, 7.8 Hz, 2H, OCH_2CH$_3$), 1.33 (t, *J* = 7.1 Hz, 3H, NCH$_2$CH_3), 1.13 (t, *J* = 7.0 Hz, 3H, OCH$_2$CH_3), 1.06 (t, *J* = 7.0 Hz, 3H, OCH$_2$CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 192.2, 162.0, 161.9, 158.6, 143.8, 140.3, 140.3, 128.8, 127.8, 127.1, 124.2, 122.7, 122.6, 121.1, 111.7, 110.1, 109.9, 62.7, 52.8, 51.8, 37.9, 16.6, 14.2, 14.2; ppm; ^{31}P NMR (243 MHz, DMSO-d_6) δ 22.4 ppm; HRMS calcd. for $C_{24}H_{27}N_4O_4P$ $[M+Na]^+$, 489.1668; found, 489.1667.

3 Antifungal activities

Table S1 *In vitro* antifungal data for MIC (μg/mL) of pyrimidines **2–8** [d,e]

Compds	Fungi				
	C. A.	*C. A.* 90023	*C. T.*	*A. F.*	*C. P.* 22019
2a	256	256	256	64	128
2b	128	64	64	128	32
2c	32	32	64	64	32
3	128	128	64	512	16
4a	128	64	256	128	128
4b	64	8	64	64	16
4c	64	256	32	256	64
4d	128	8	256	32	256
4e	128	128	256	64	256
4f	128	128	256	16	64
4g	128	256	256	64	128
5a	256	64	256	256	128
5b	256	64	256	256	128
5c	128	128	128	256	64
6a	64	64	128	256	16
6b	256	128	64	256	16
7	128	256	256	128	256
8	256	512	512	32	512
C	4	2	8	256	4

[d] *C. A.*, *Candida albicans*; *C. A.* 90023, *Candida albicans* ATCC 90023; *C. T.*, *Candida tropicals*; *A. F.*, *Aspergillus fumigatus*; *C. P.* 22019, *Candida parapsilosis* ATCC 22019. [e] **C** = Fluconazole

4 Molecular docking study of compound 2c

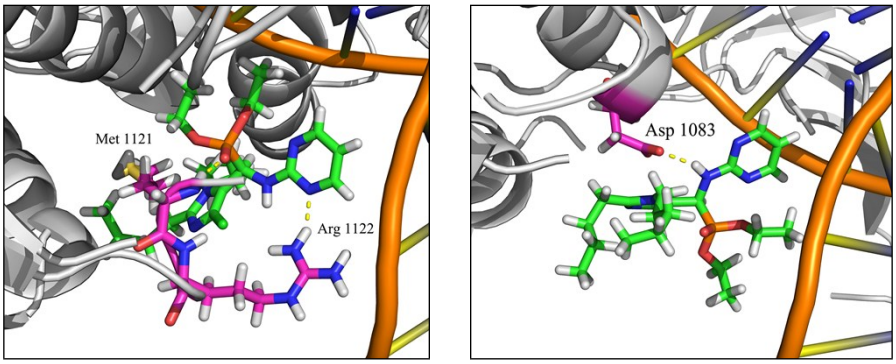


Figure S1. Three-dimensional conformation of compound **2c** docked in bacterial DNA-gyrase complex (PDB code: 2XCS).

5 Absorption spectra of NR interaction with MRSA DNA

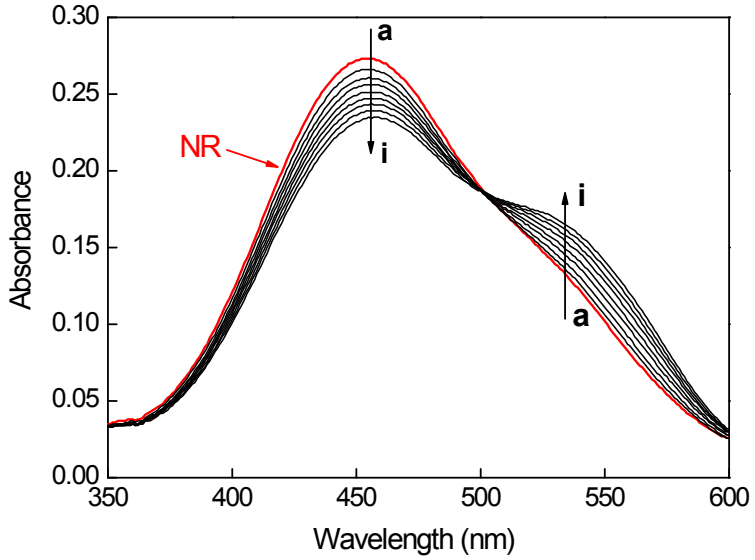
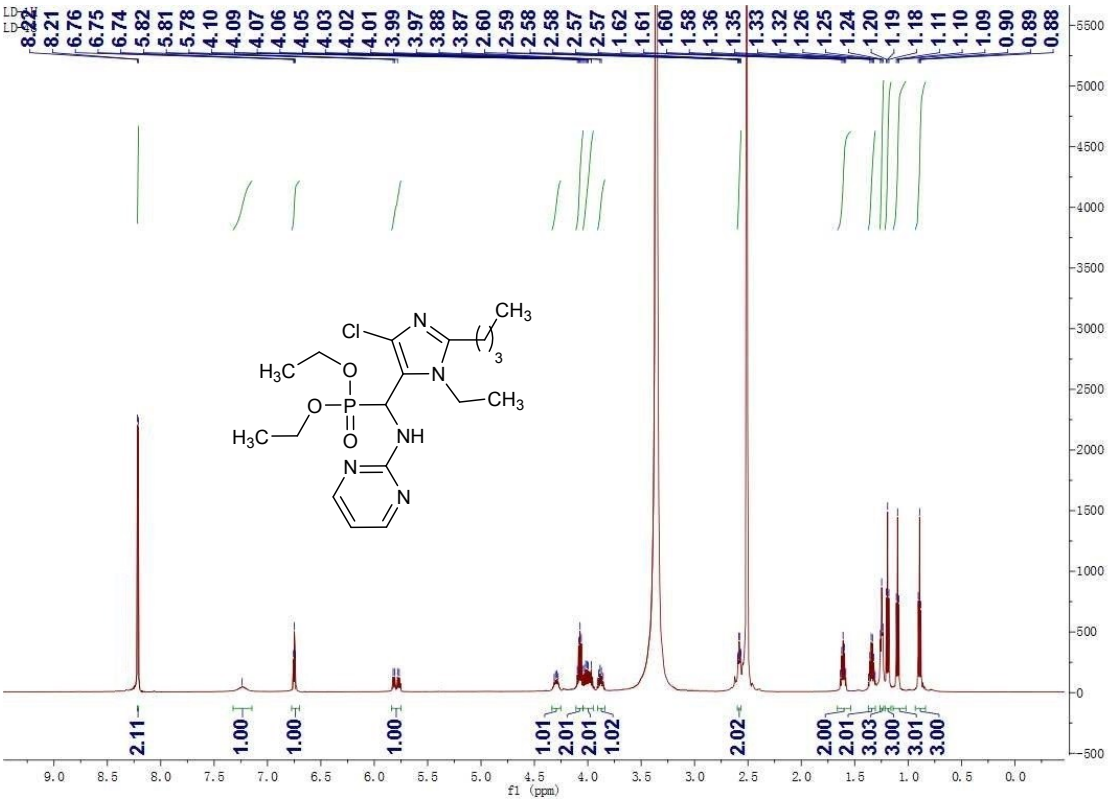


Figure S2. UV absorption spectra of NR in the presence of DNA (pH = 7.4, T = 287 K). c (NR) = 2 × 10^{-5} mol/L, and c (DNA) = 0–1.49 × 10^{-5} mol/L for curves *a*–*i* respectively at an increment of 0.19 × 10^{-5}.

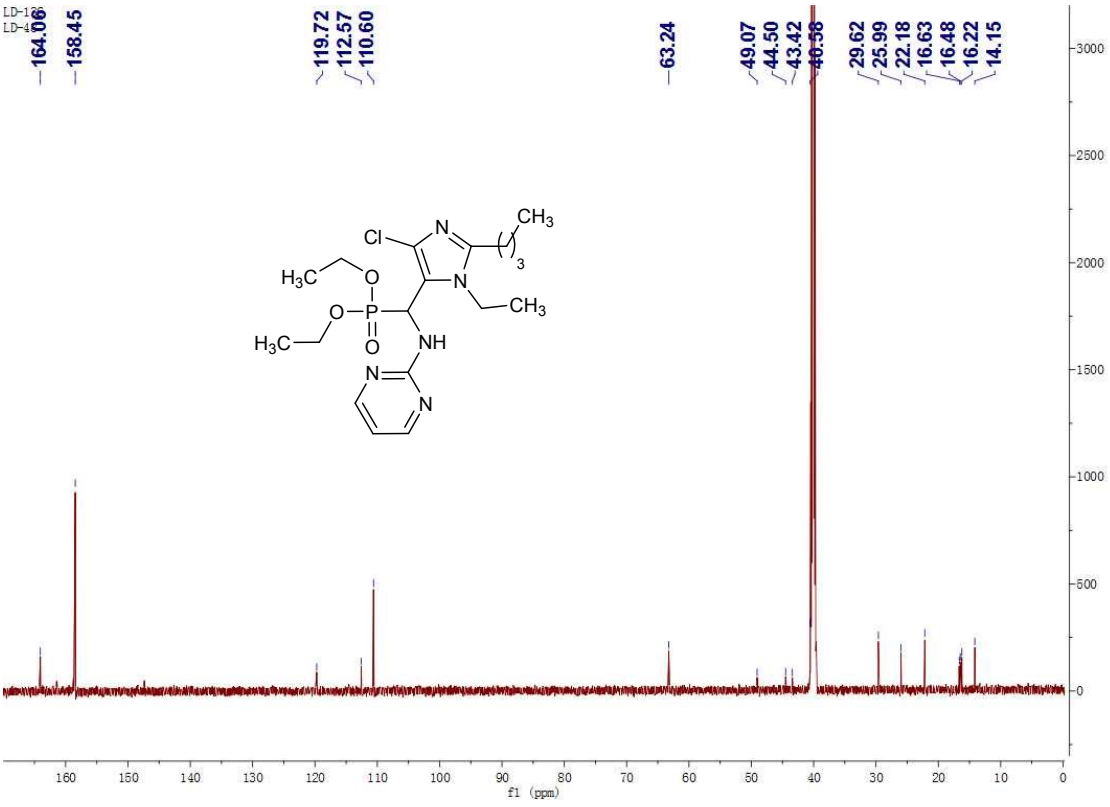
6 Some representative spectra for compounds' structure

6.1 Spectra of compound 2a

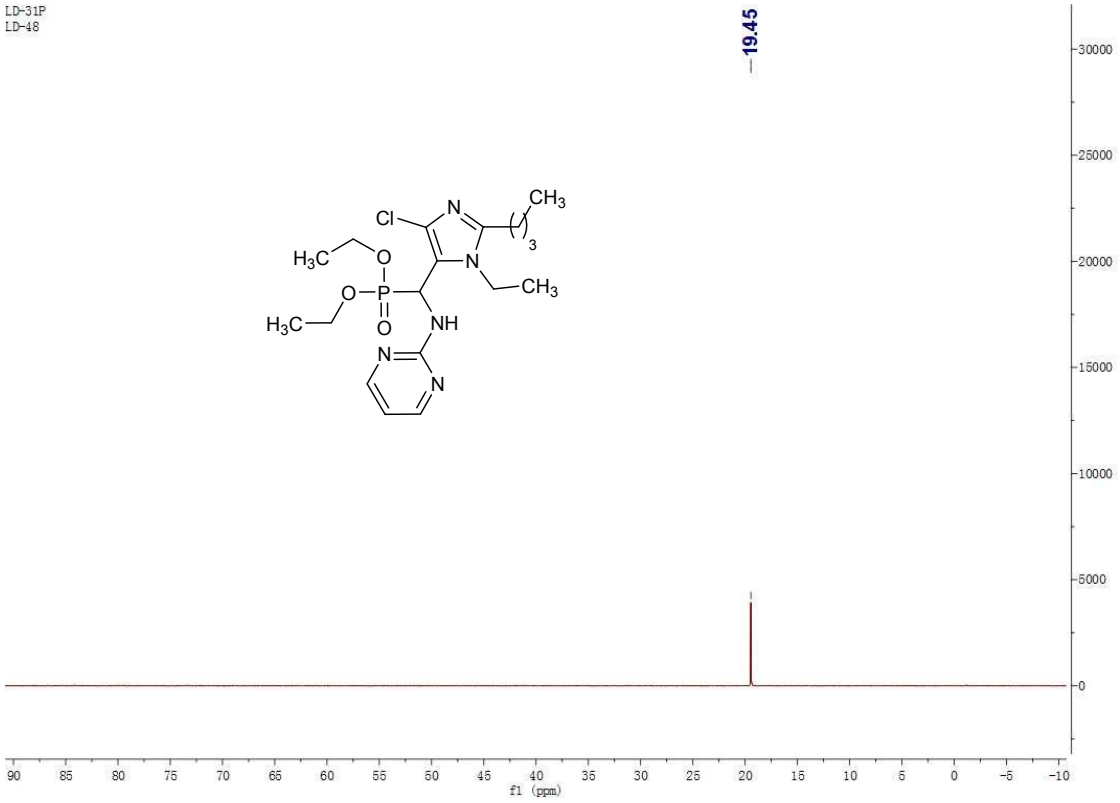
^{1}H NMR Spectrum



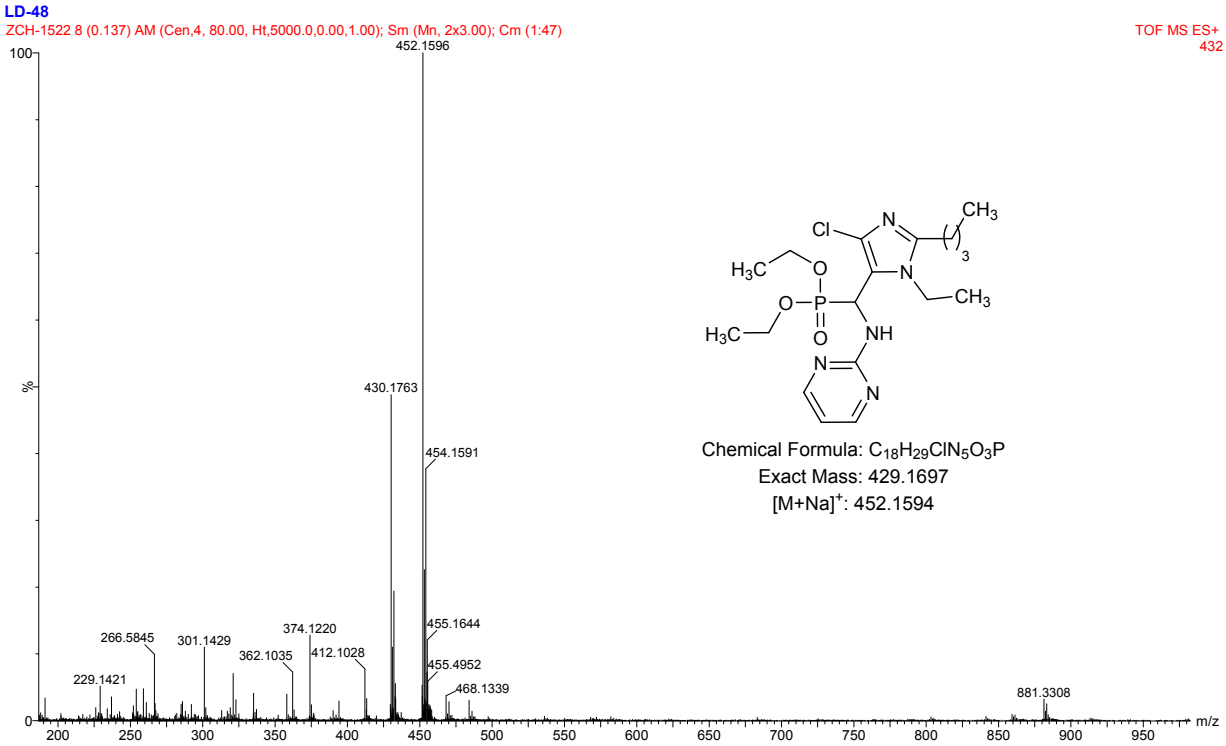
^{13}C NMR Spectrum



^{31}P NMR Spectrum

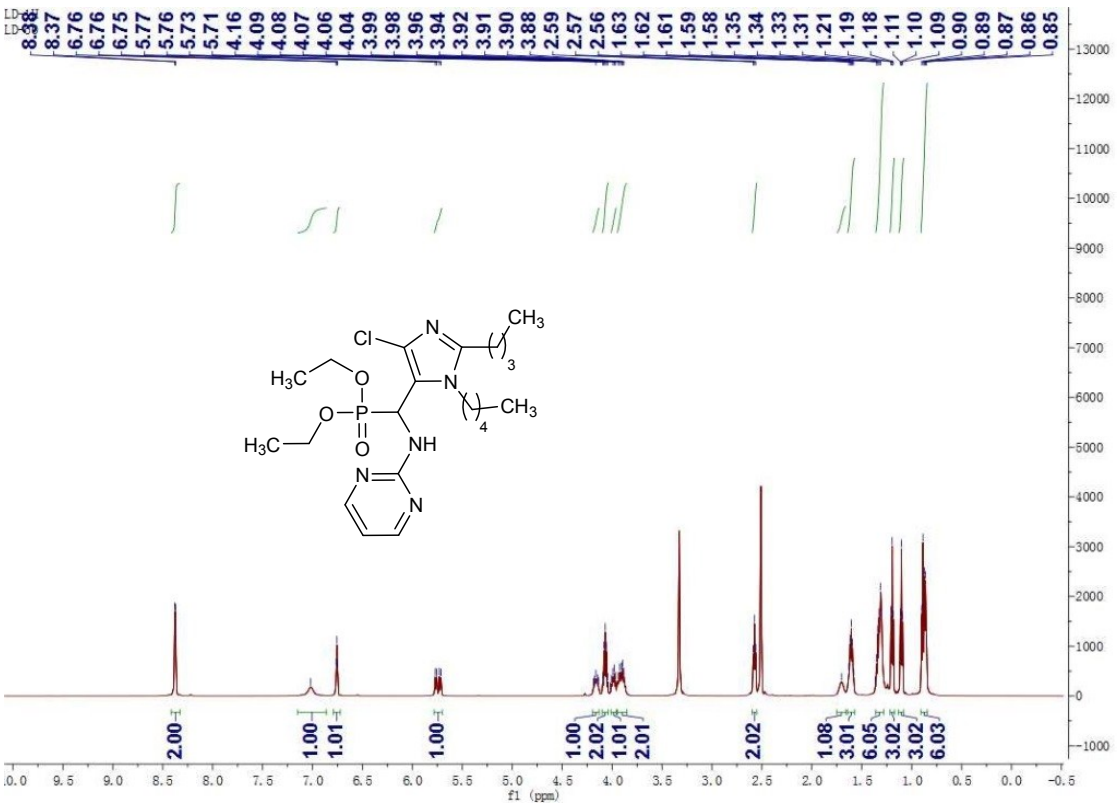


HRMS Spectrum

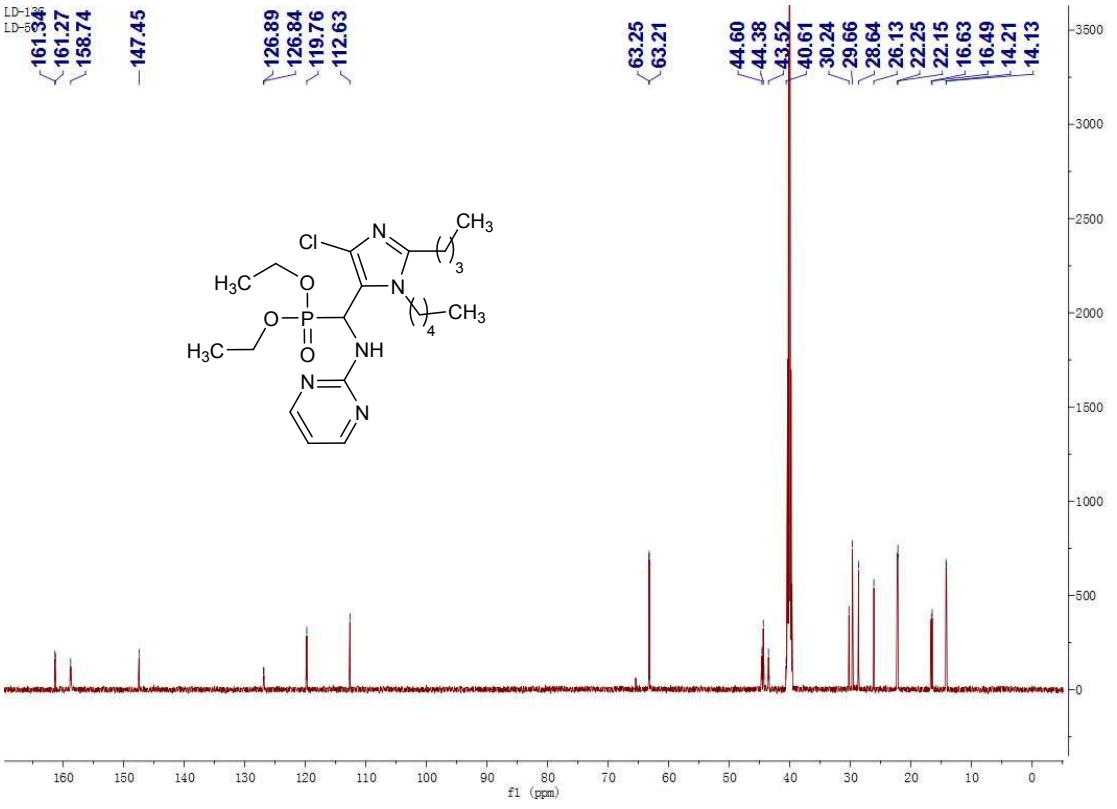


6.2 Spectra of compound 2c

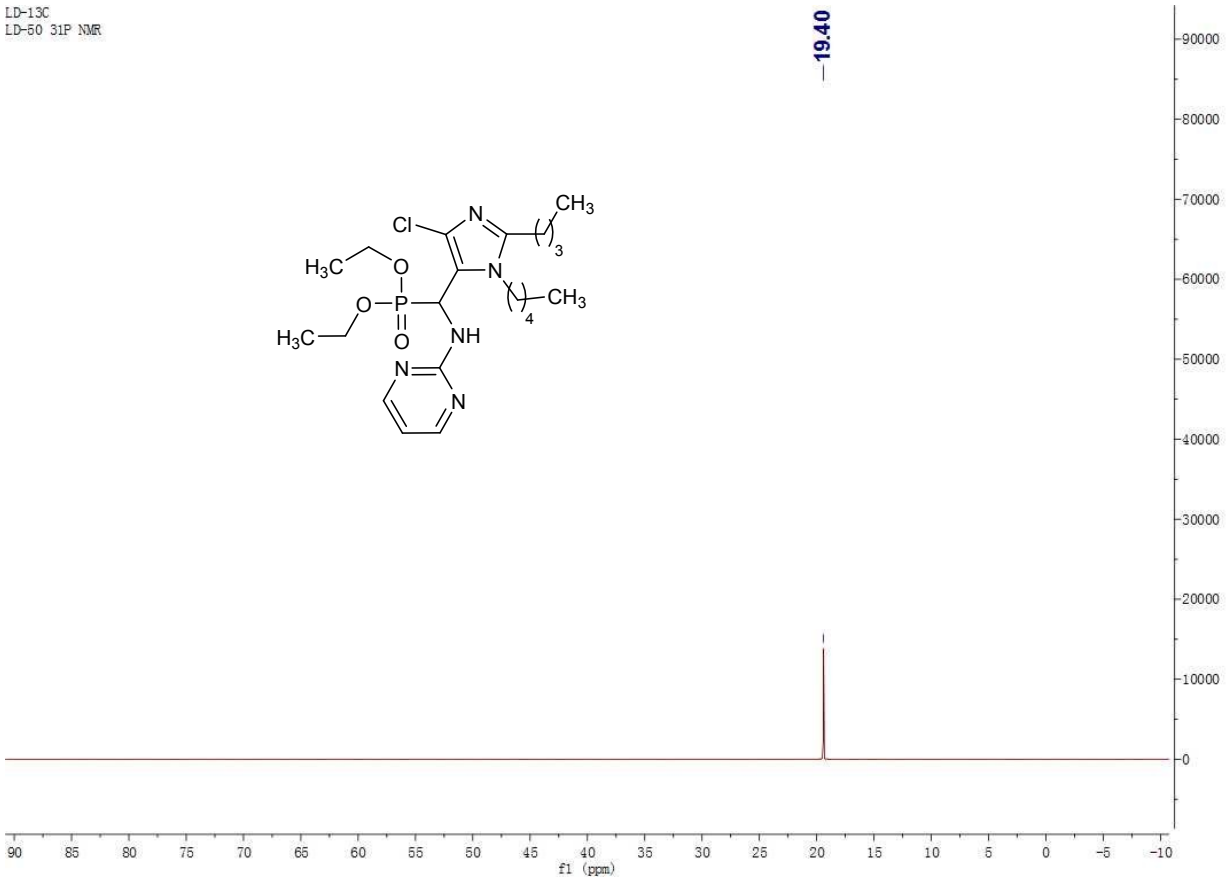
^{1}H NMR Spectrum



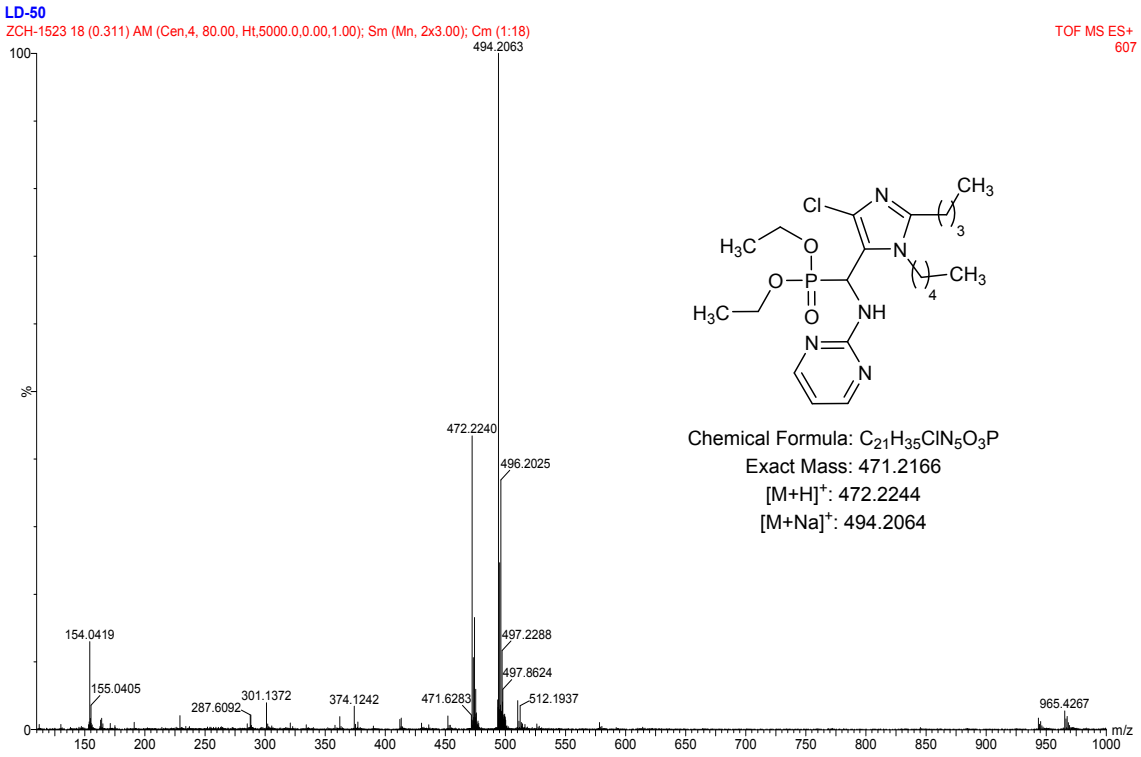
^{13}C NMR Spectrum



^{31}P NMR Spectrum



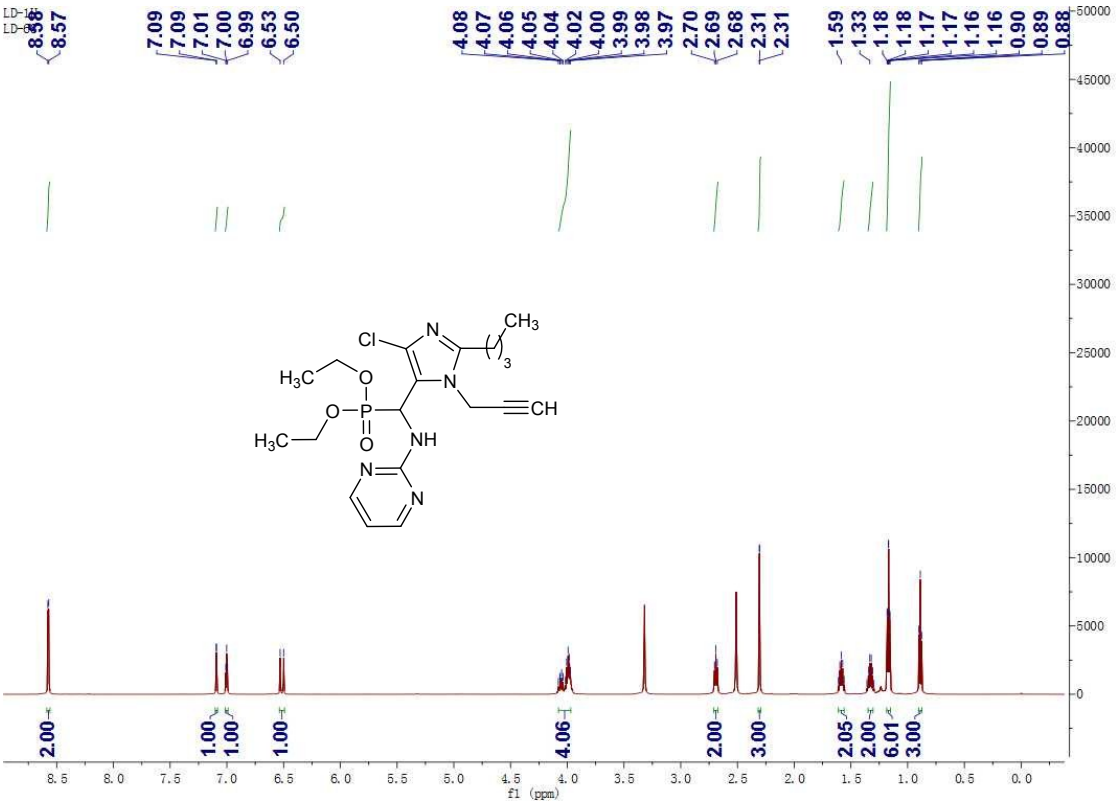
HRMS Spectrum



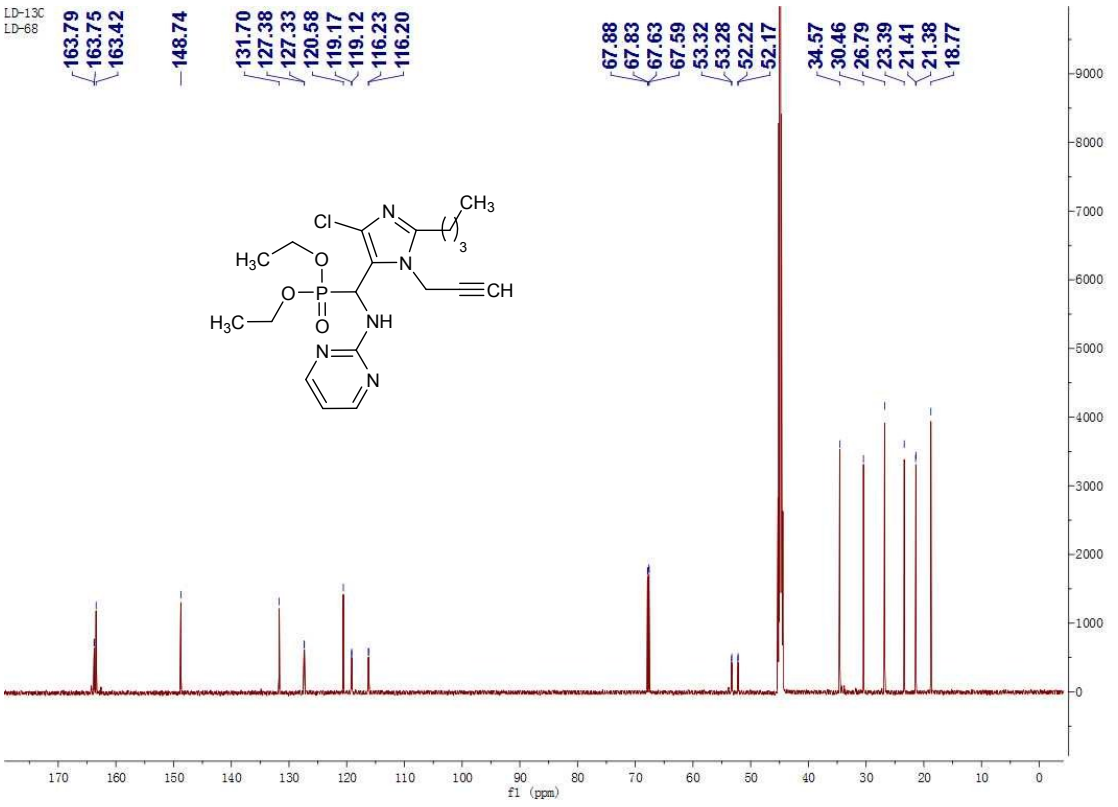
Chemical Formula: $C_{21}H_{35}ClN_5O_3P$
Exact Mass: 471.2166
$[M+H]^+$: 472.2244
$[M+Na]^+$: 494.2064

6.3 Spectra of compound 3

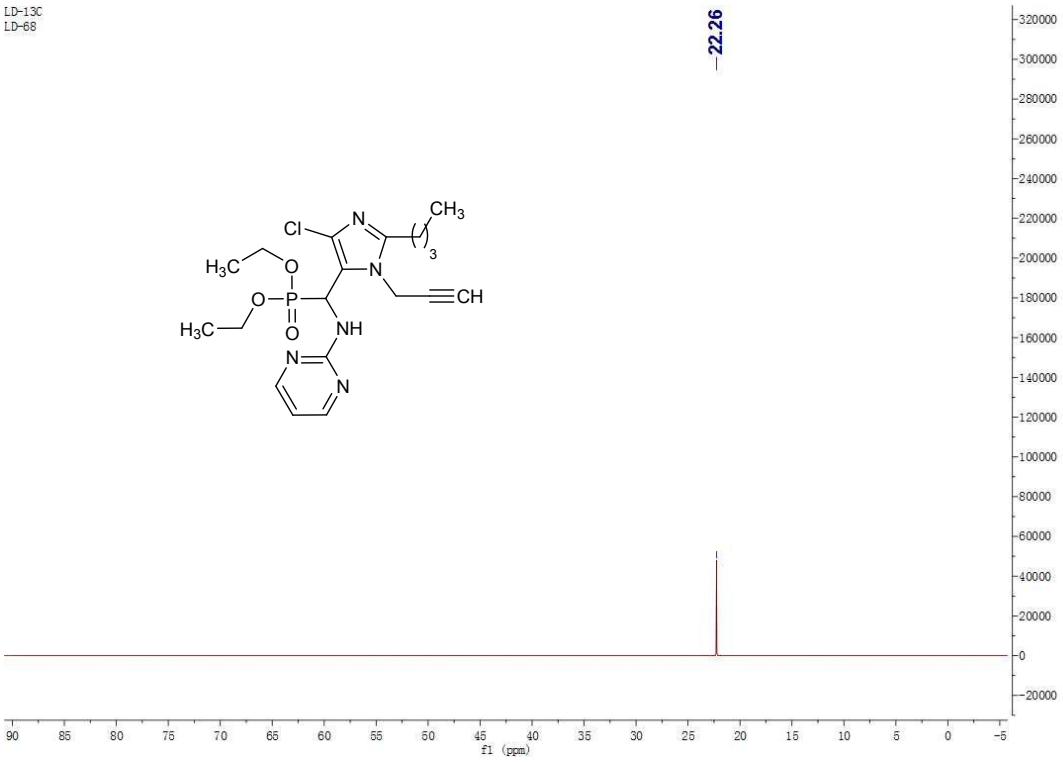
^{1}H NMR Spectrum



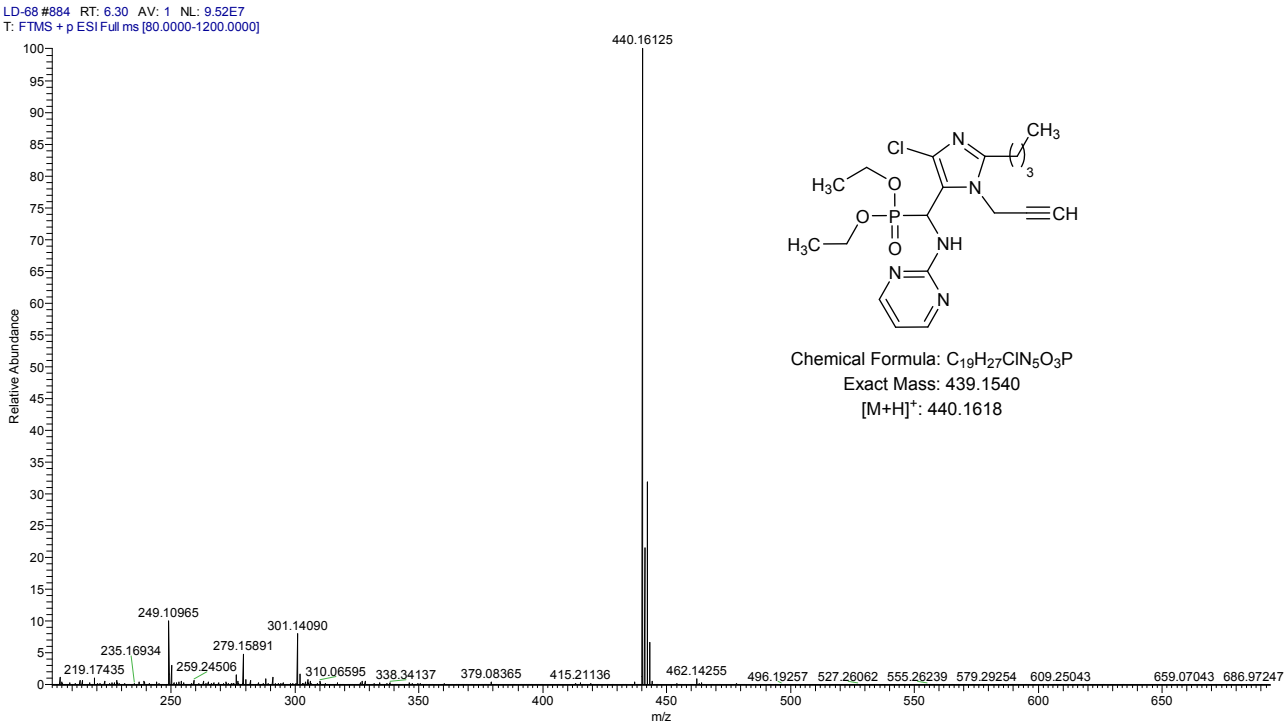
^{13}C NMR Spectrum



^{31}P NMR Spectrum



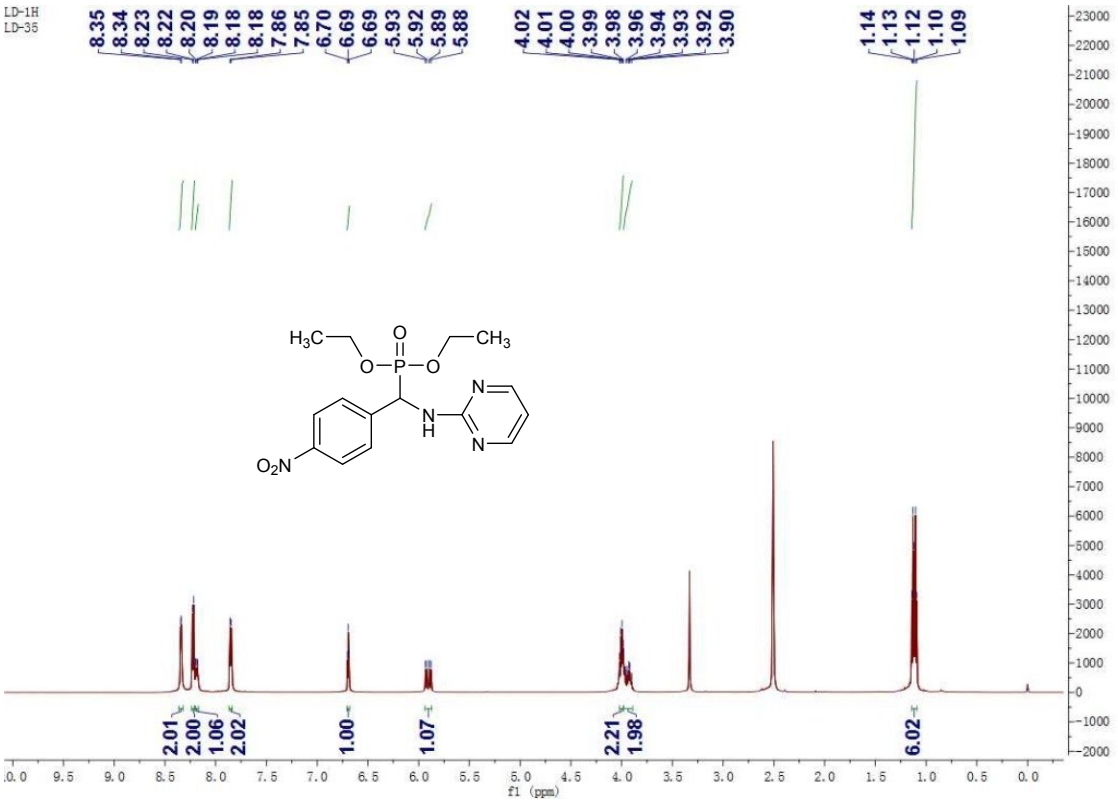
HRMS Spectrum



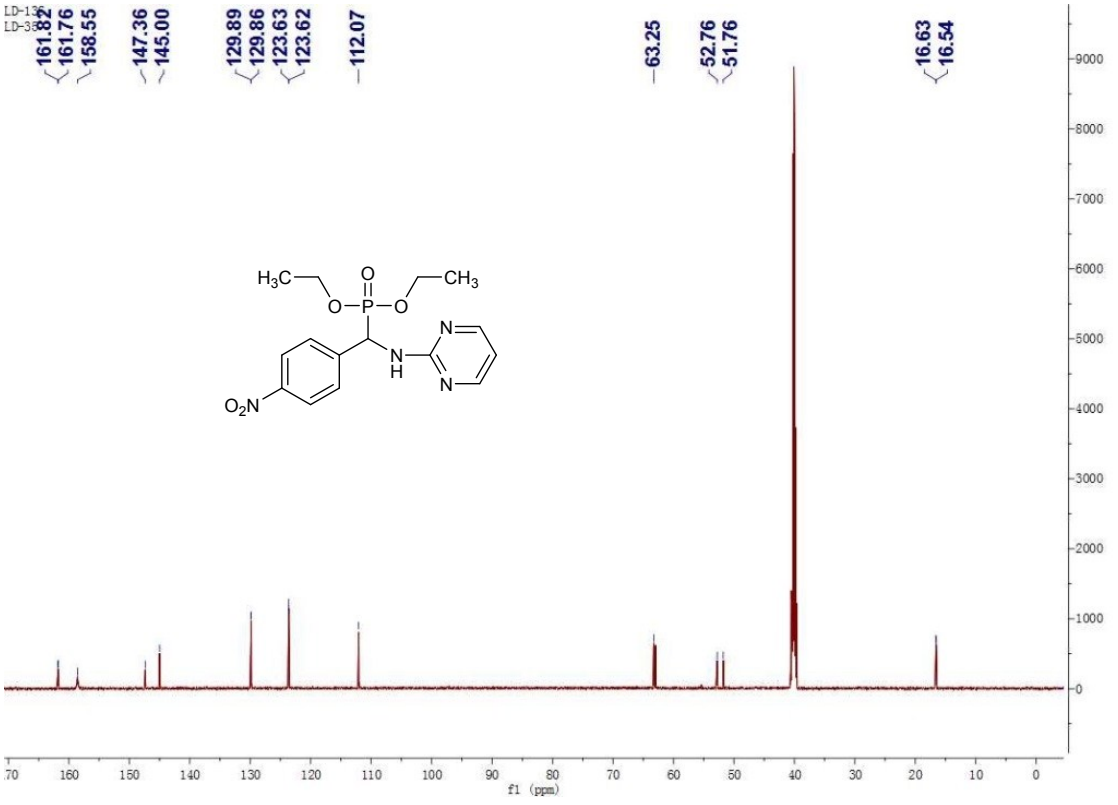
Chemical Formula: $C_{19}H_{27}ClN_5O_3P$
Exact Mass: 439.1540
$[M+H]^+$: 440.1618

6.4 Spectra of compound 4a

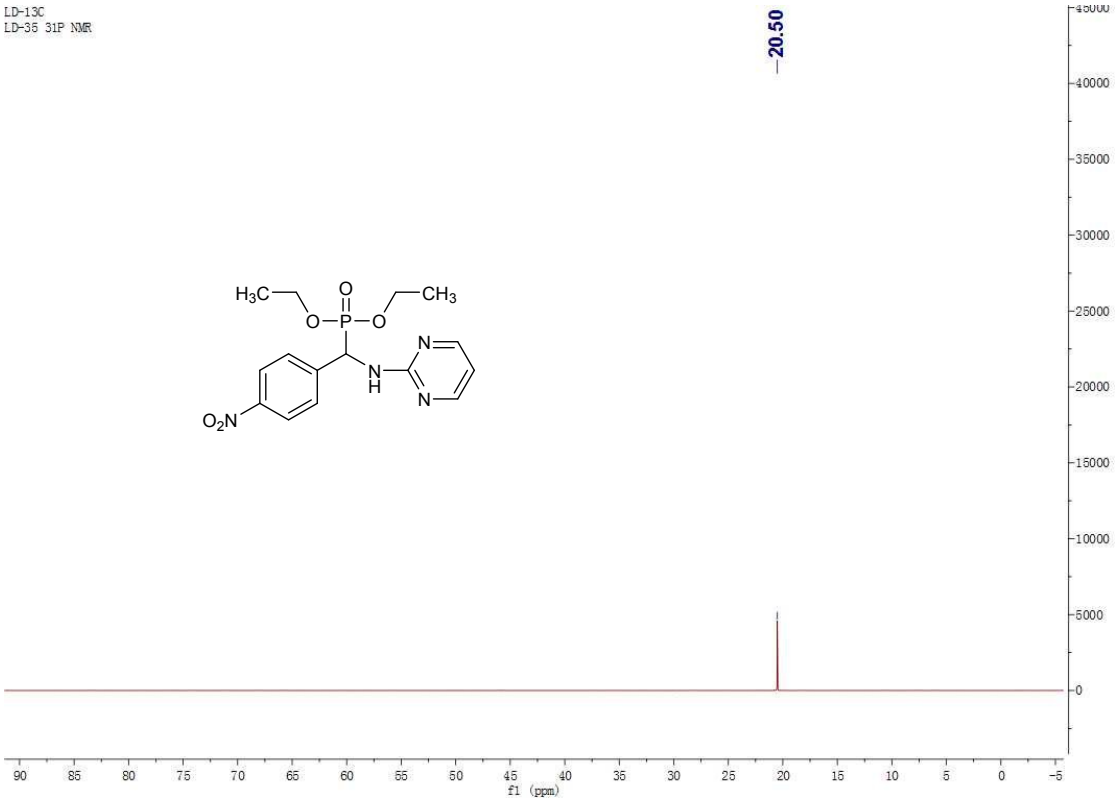
^{1}H NMR Spectrum



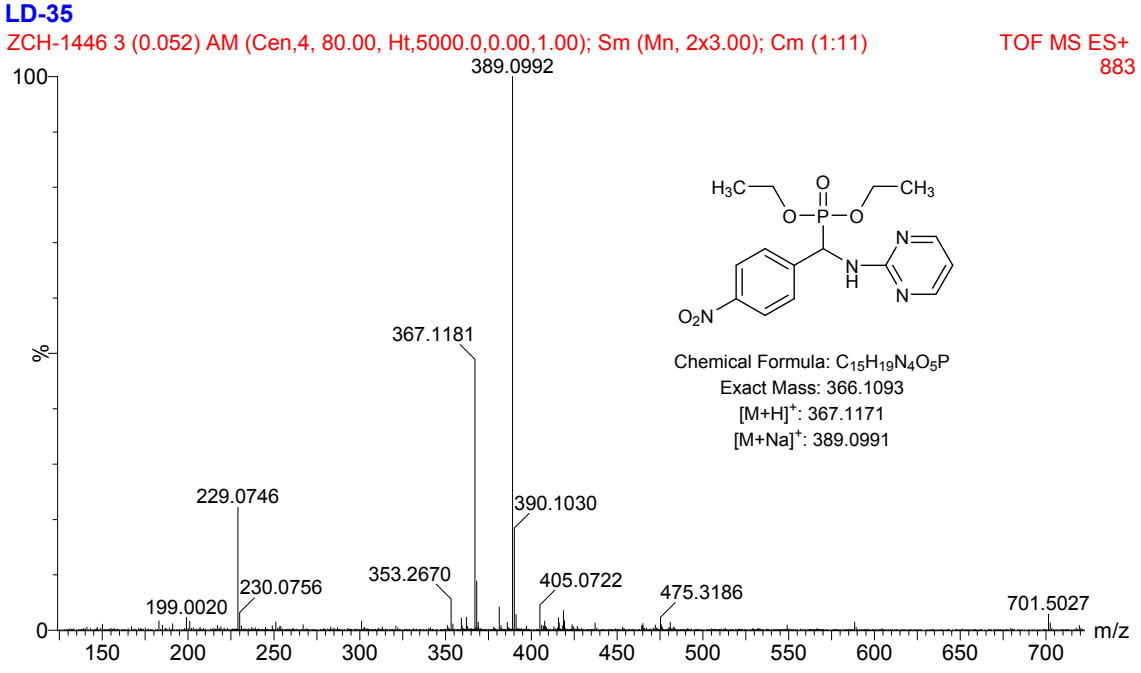
^{13}C NMR Spectrum



^{31}P NMR Spectrum

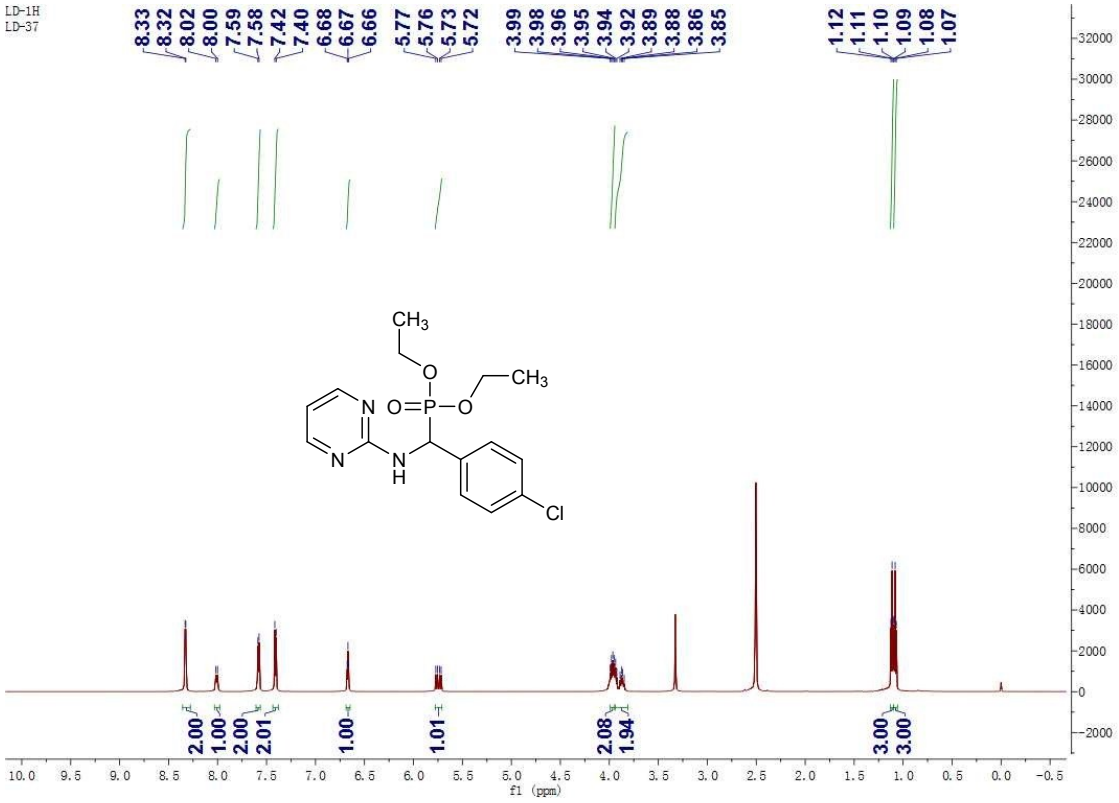


HRMS Spectrum

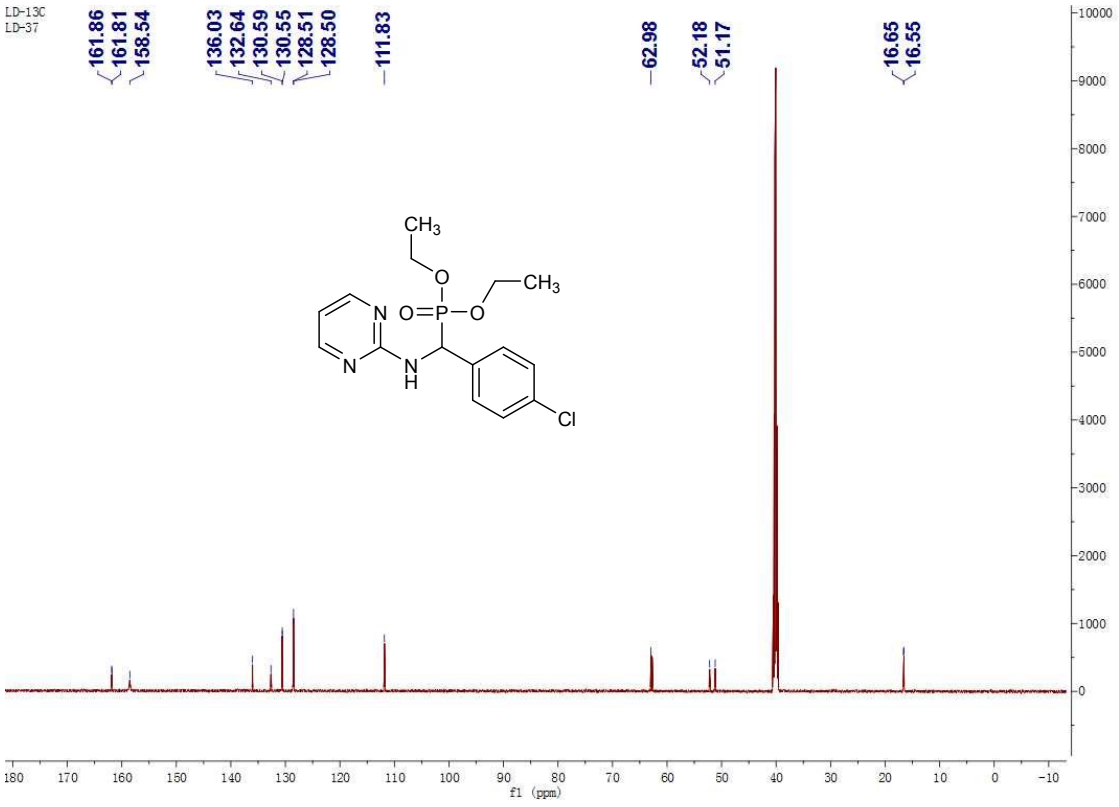


6.5 Spectra of compound 4d

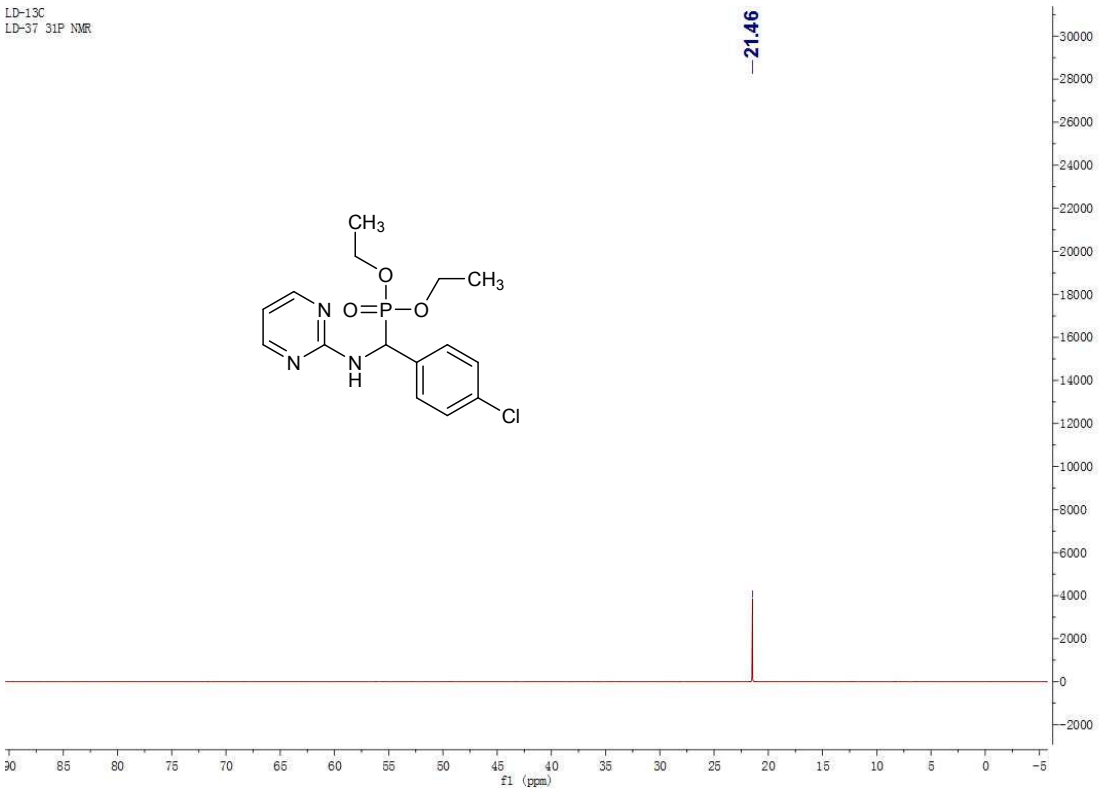
^{1}H NMR Spectrum



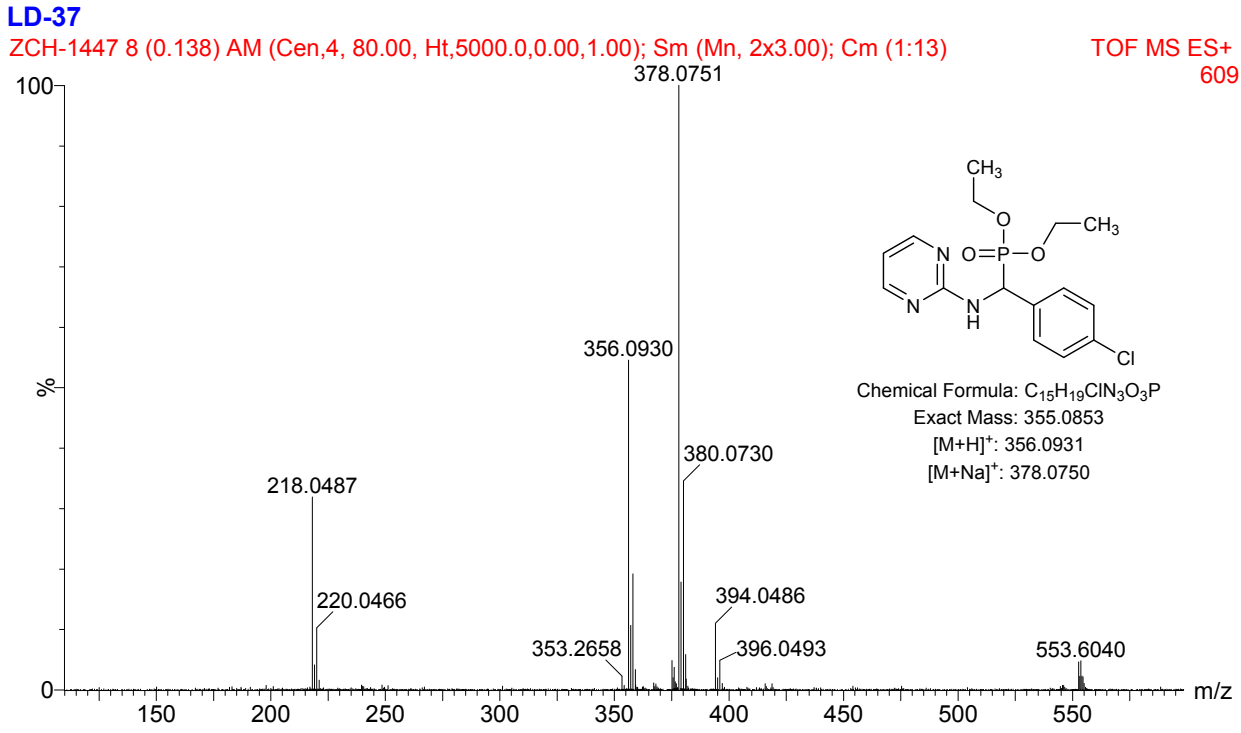
^{13}C NMR Spectrum



^{31}P NMR Spectrum

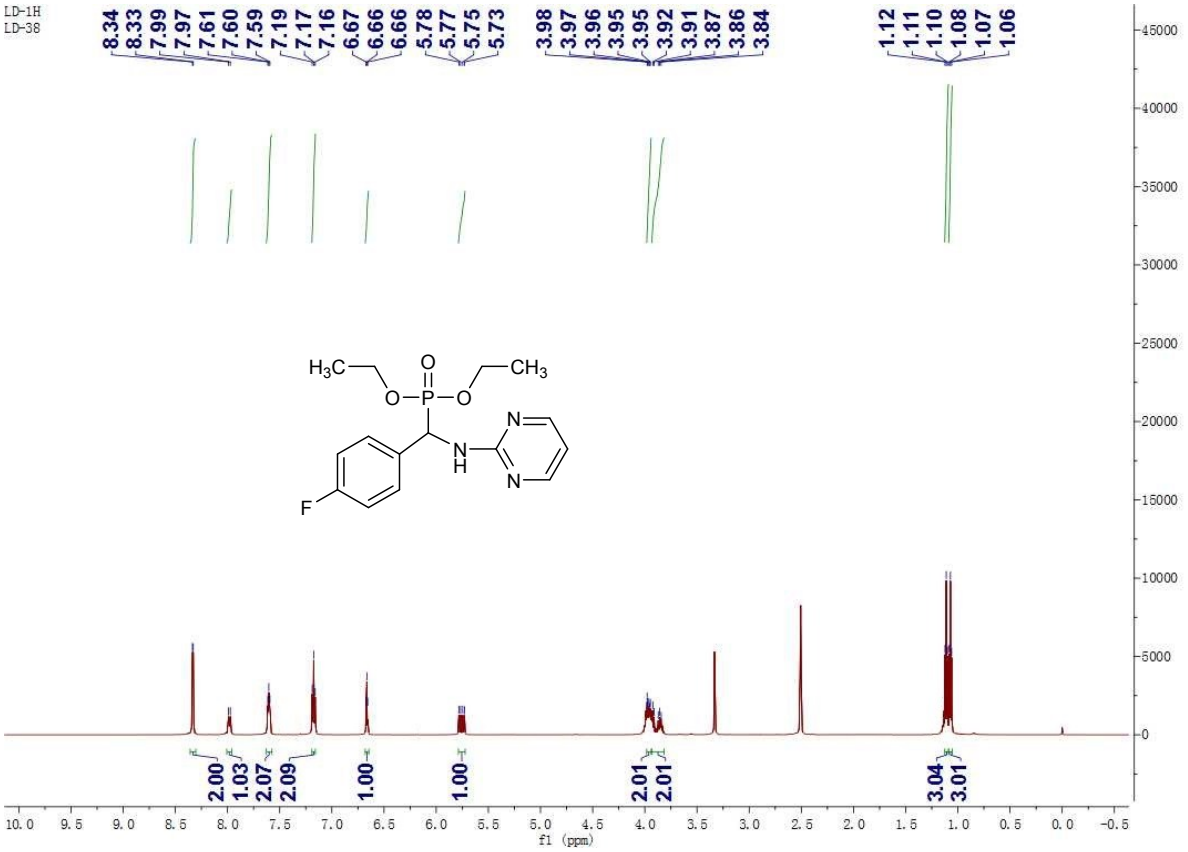


HRMS Spectrum

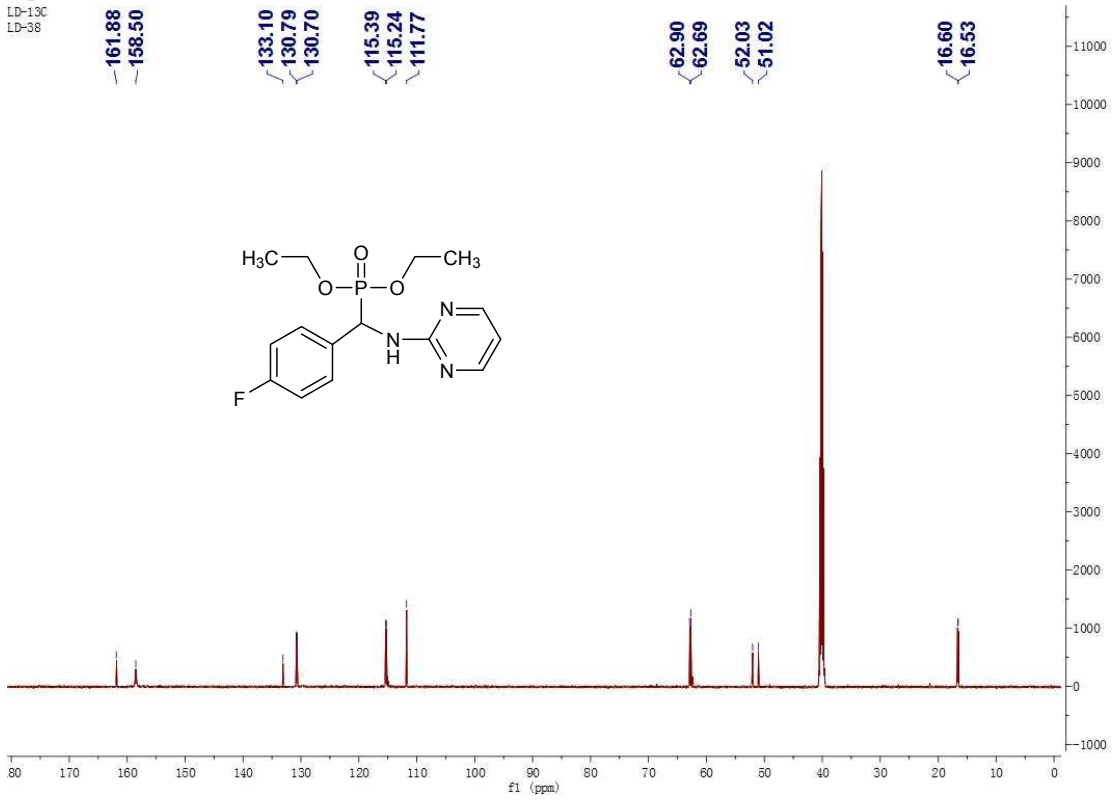


6.6 Spectra of compound 4e

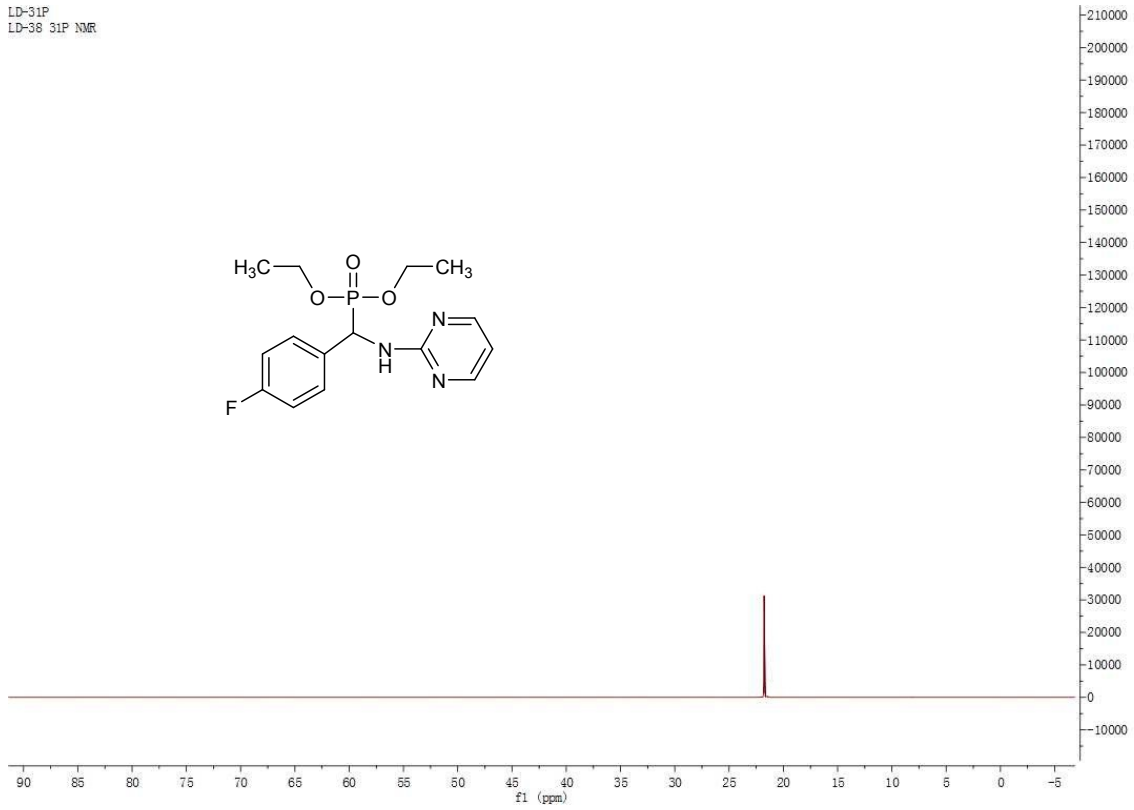
^{1}H NMR Spectrum



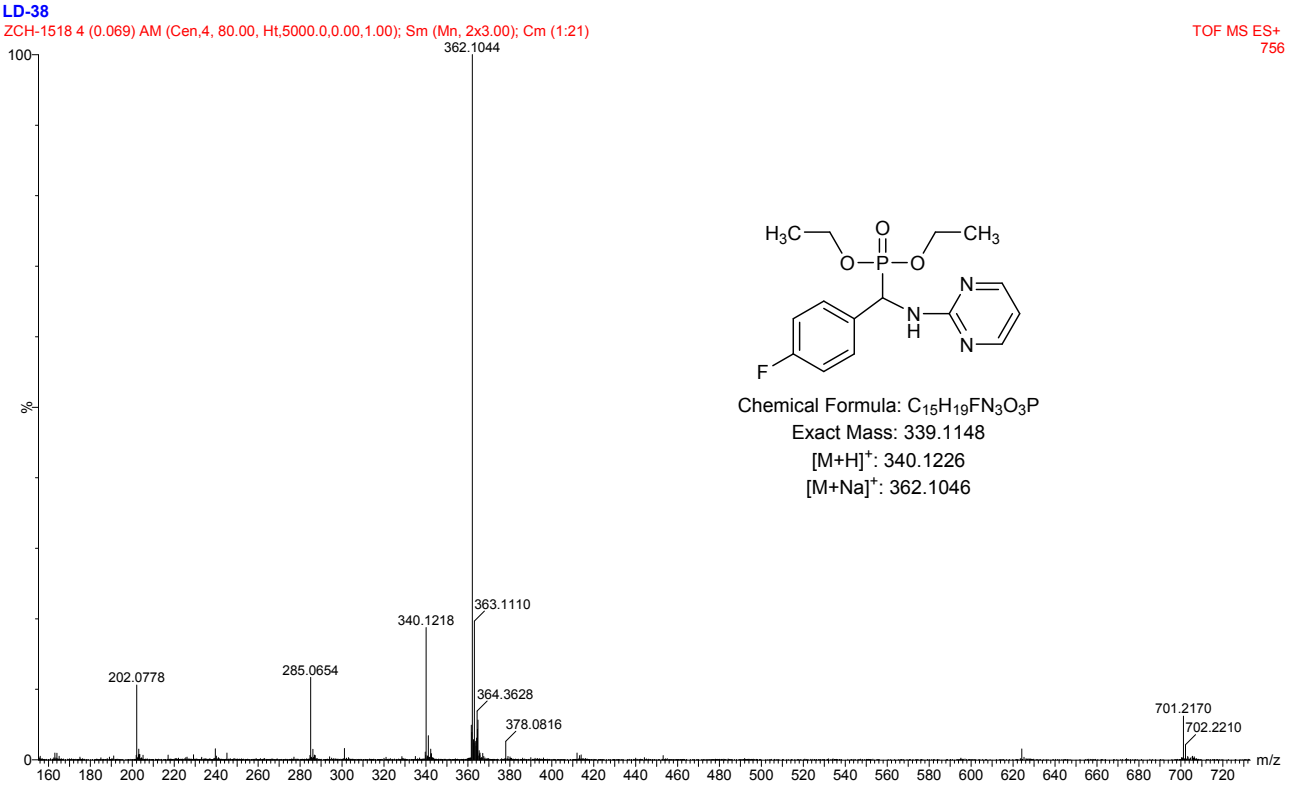
^{13}C NMR Spectrum



^{31}P NMR Spectrum



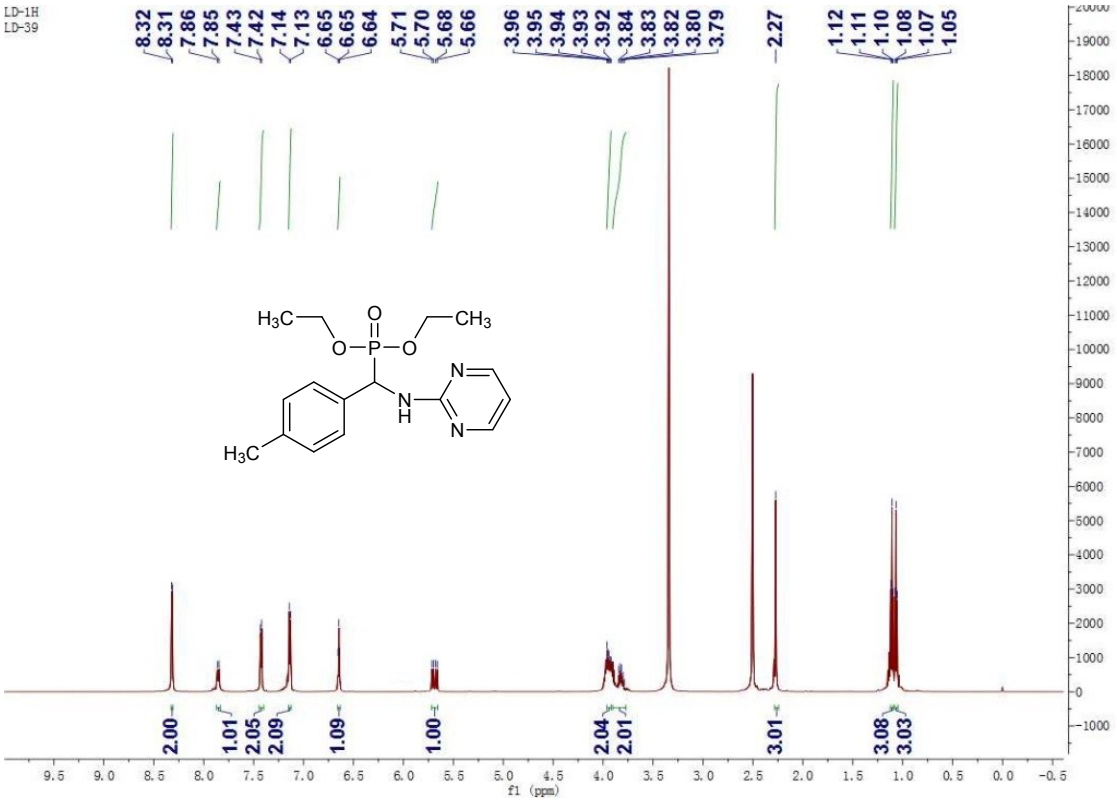
HRMS Spectrum



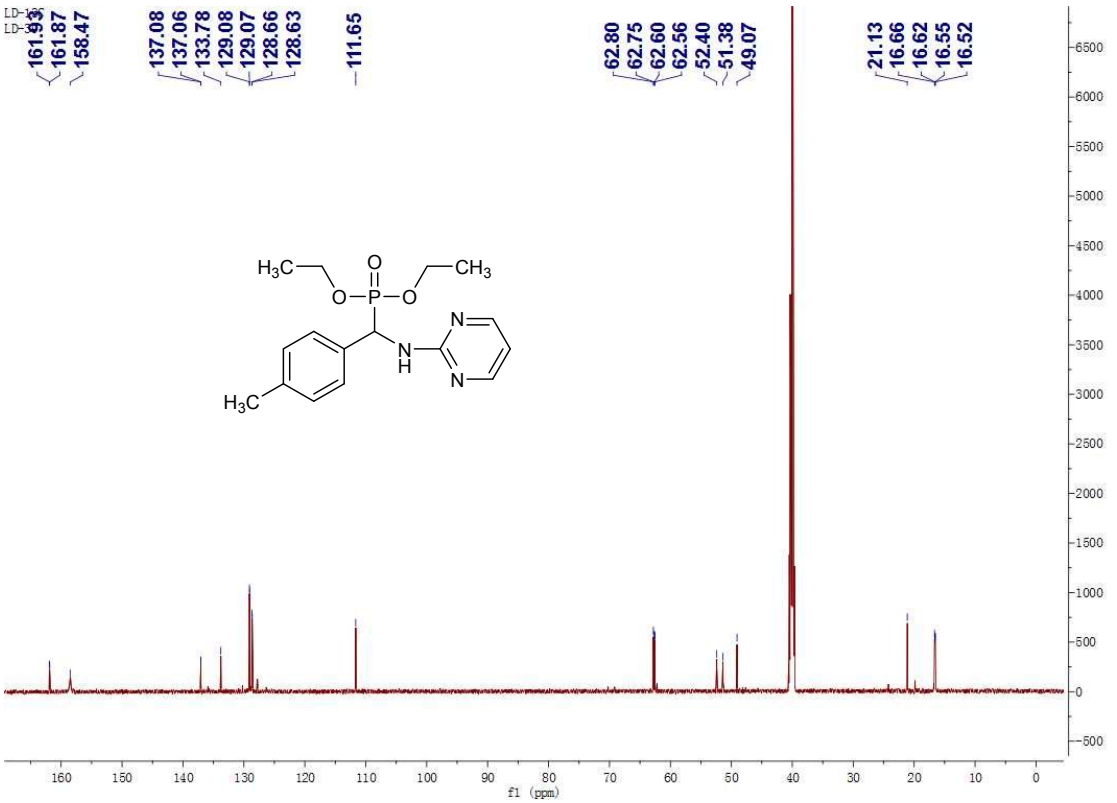
Chemical Formula: $C_{15}H_{19}FN_3O_3P$
Exact Mass: 339.1148
$[M+H]^+$: 340.1226
$[M+Na]^+$: 362.1046

6.7 Spectra of compound 4f

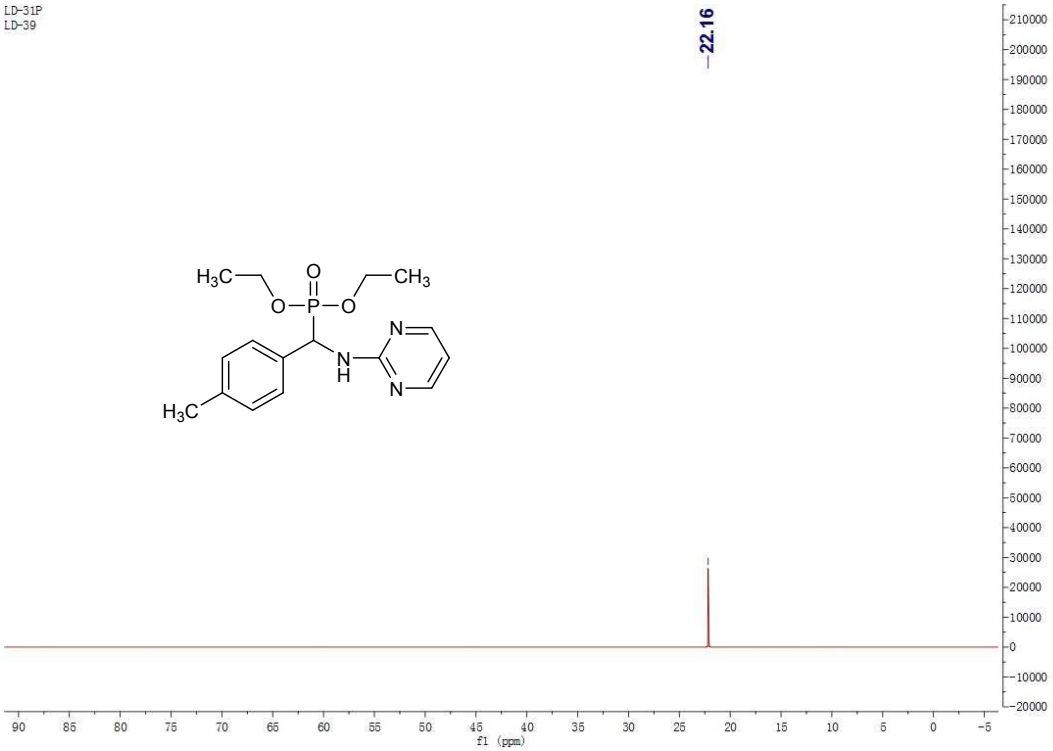
^{1}H NMR Spectrum



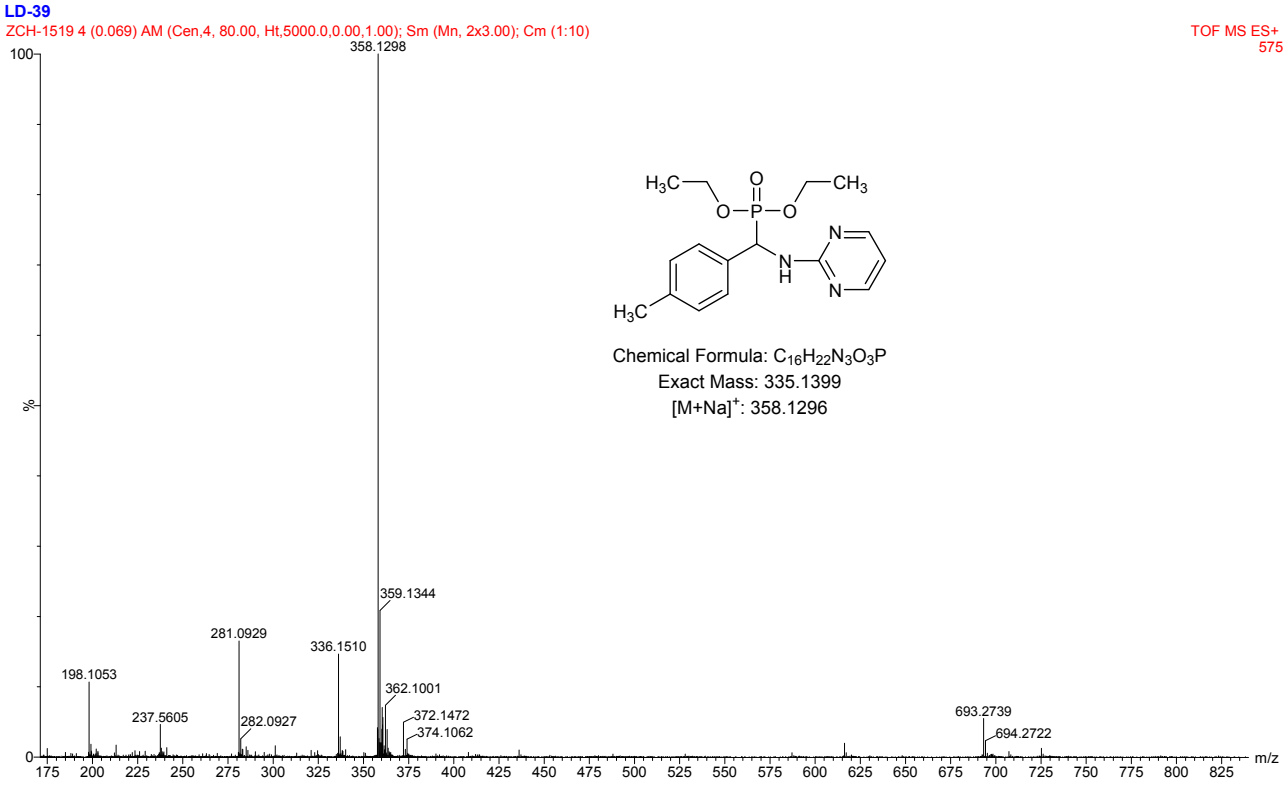
^{13}C NMR Spectrum



^{31}P NMR Spectrum



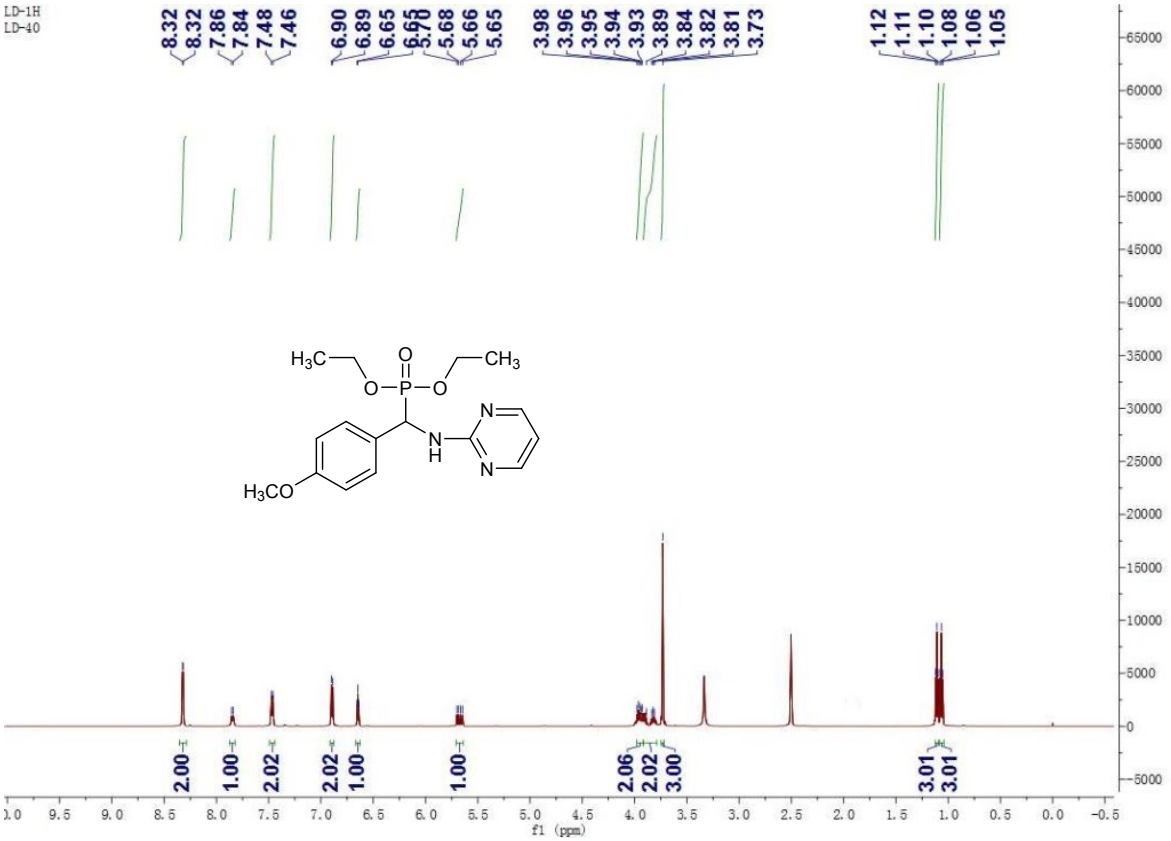
HRMS Spectrum



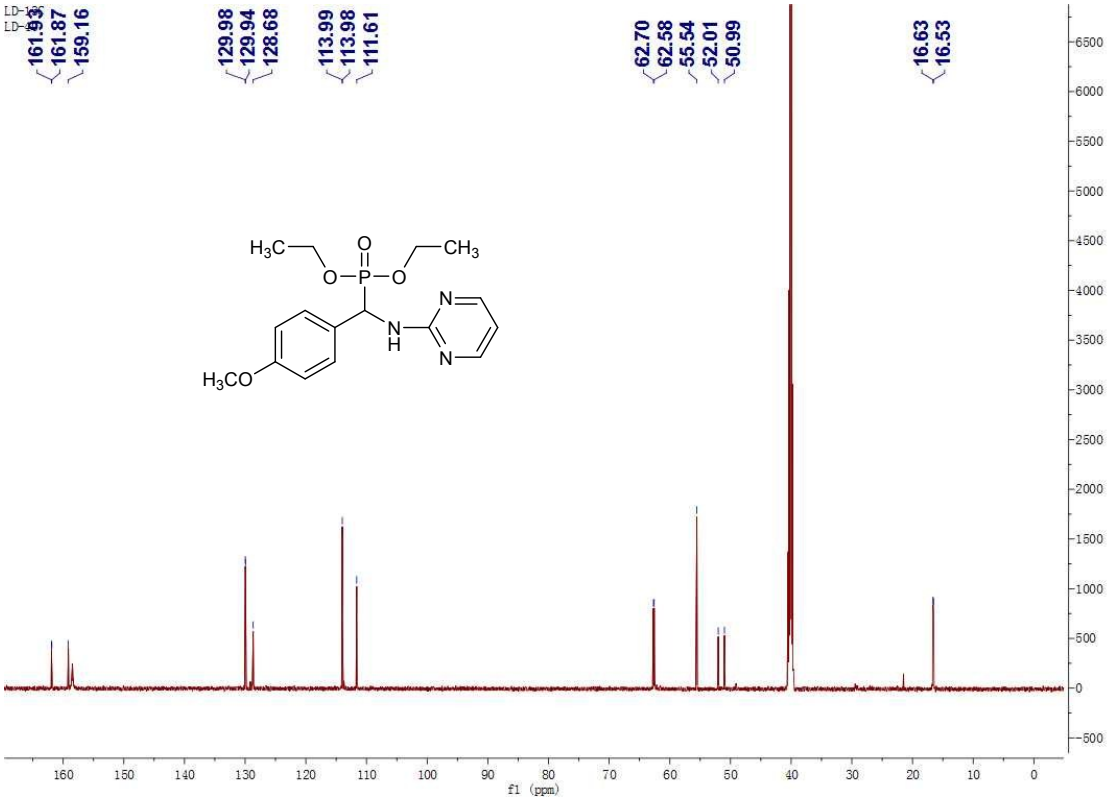
Chemical Formula: $C_{16}H_{22}N_3O_3P$
Exact Mass: 335.1399
$[M+Na]^+$: 358.1296

6.8 Spectra of compound 4g

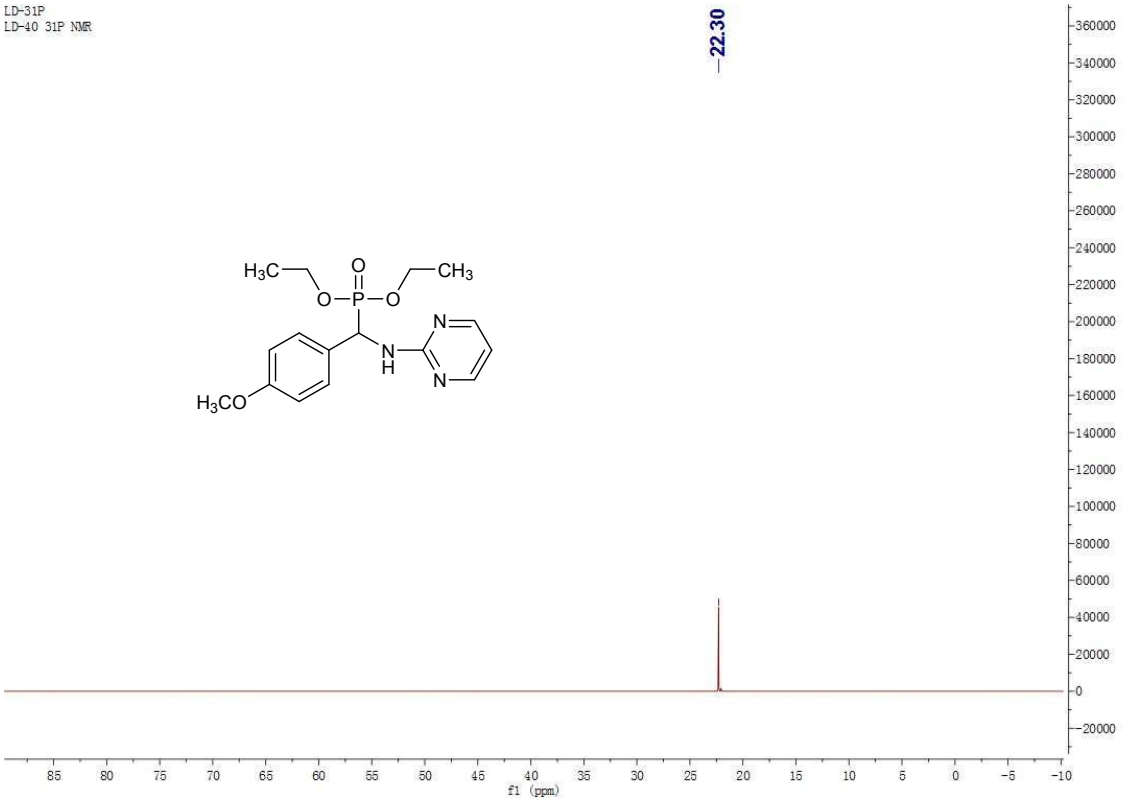
^{1}H NMR Spectrum



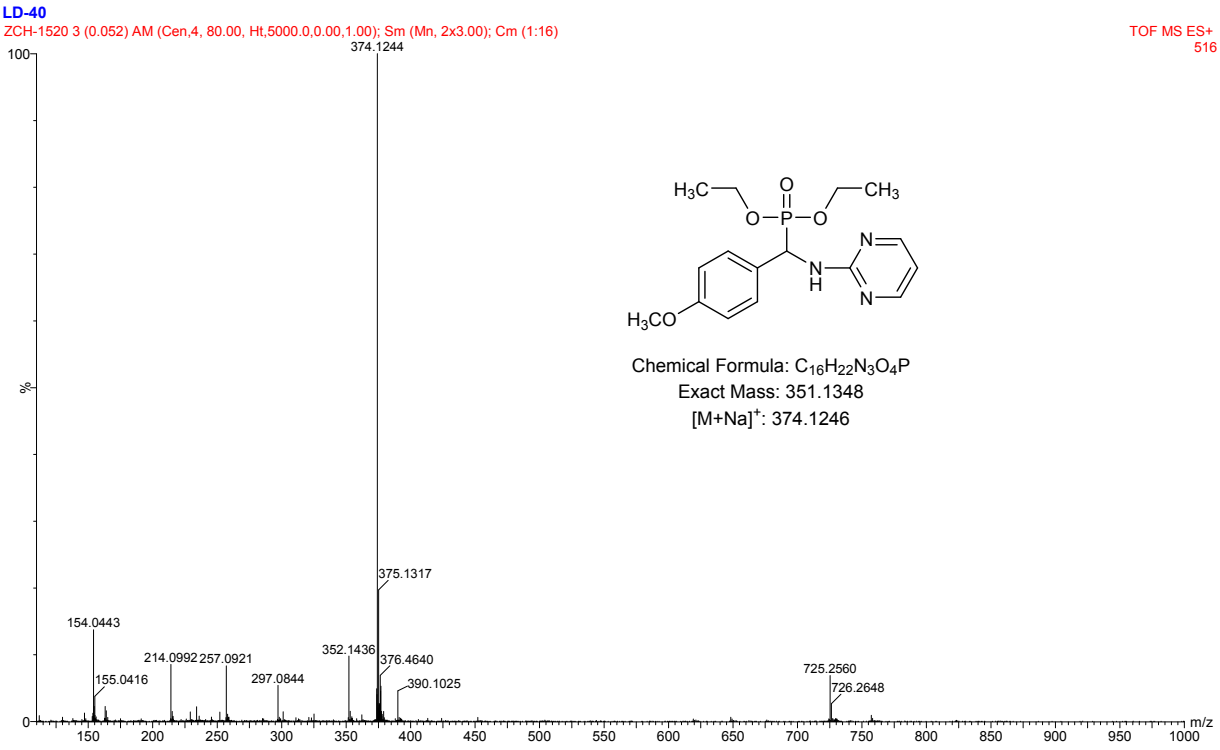
^{13}C NMR Spectrum



^{31}P NMR Spectrum



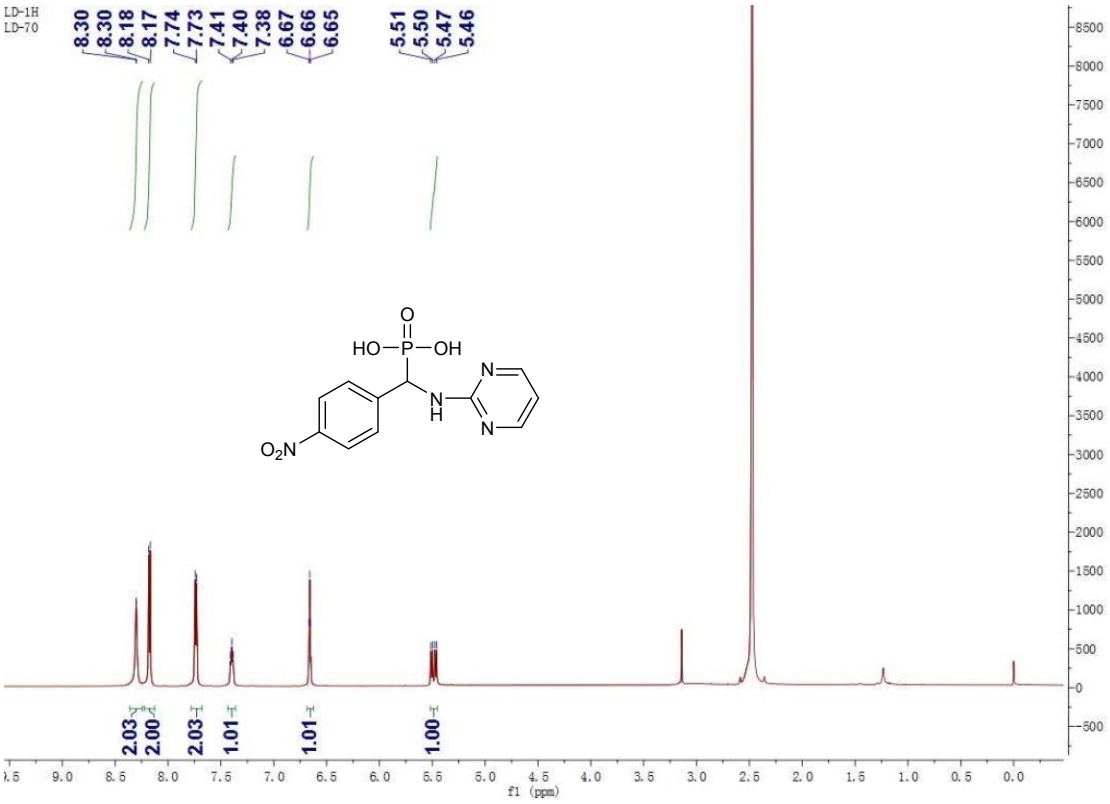
HRMS Spectrum



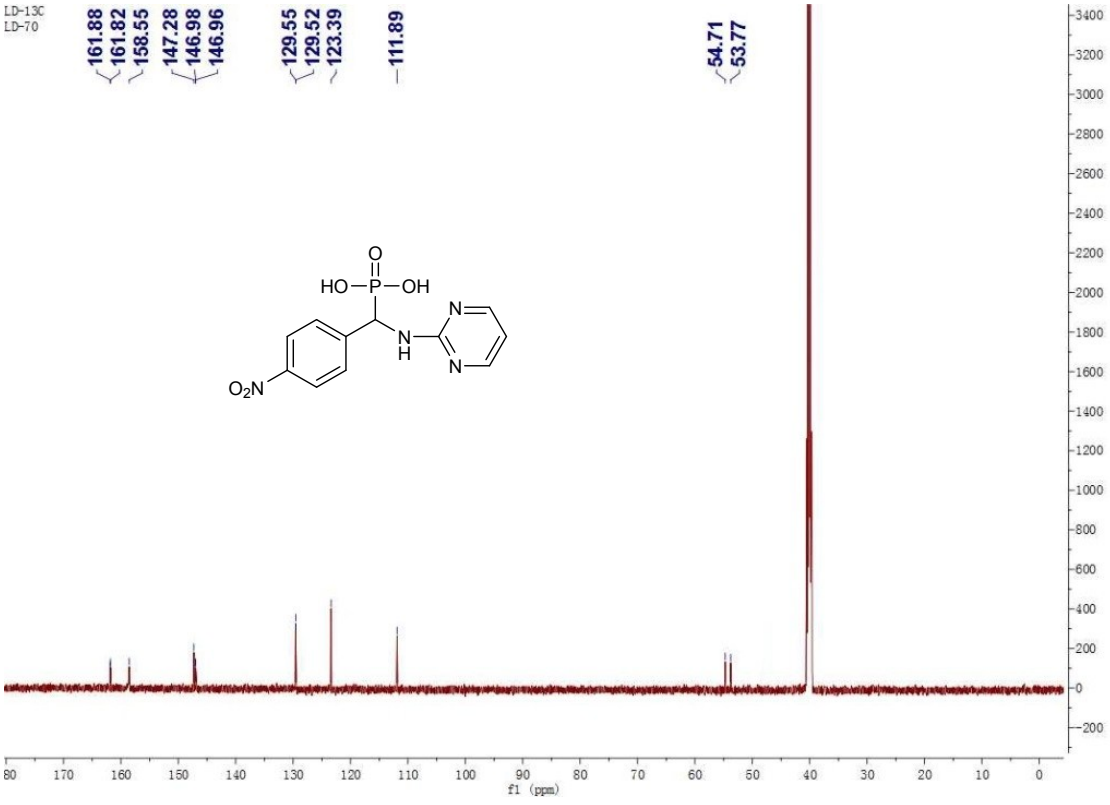
Chemical Formula: $C_{16}H_{22}N_3O_4P$
Exact Mass: 351.1348
$[M+Na]^+$: 374.1246

6.9 Spectra of compound 5a

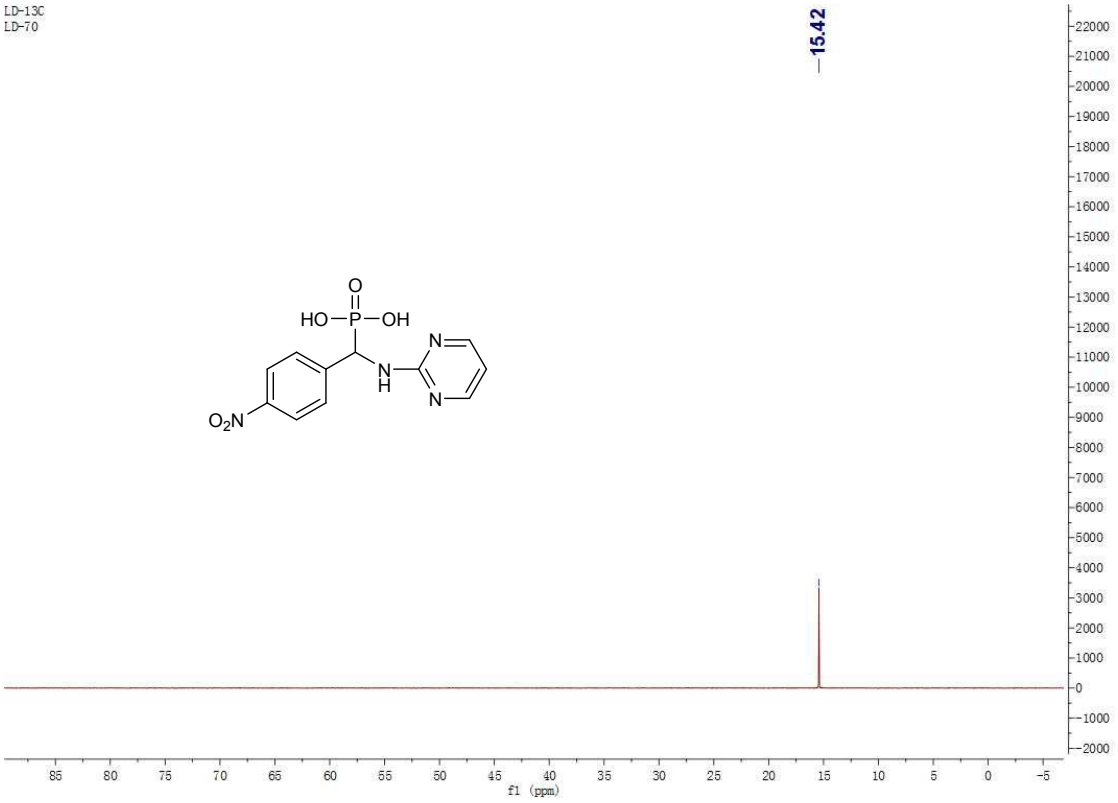
^{1}H NMR Spectrum



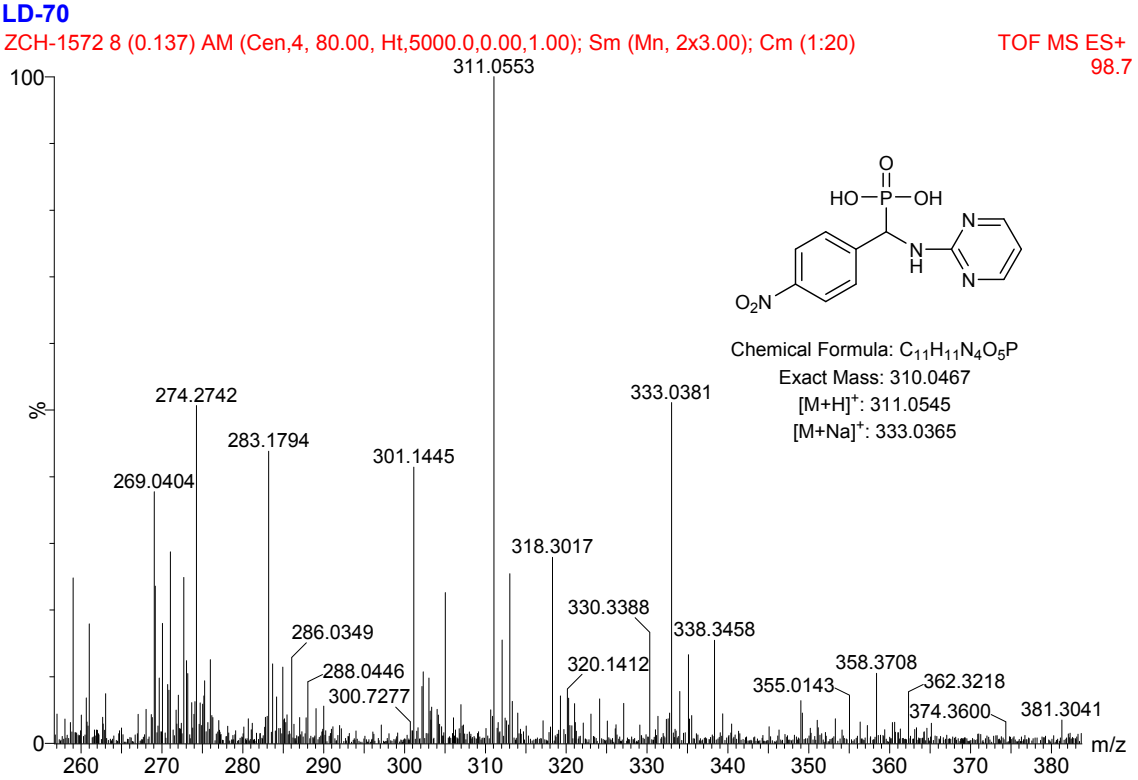
^{13}C NMR Spectrum



^{31}P NMR Spectrum

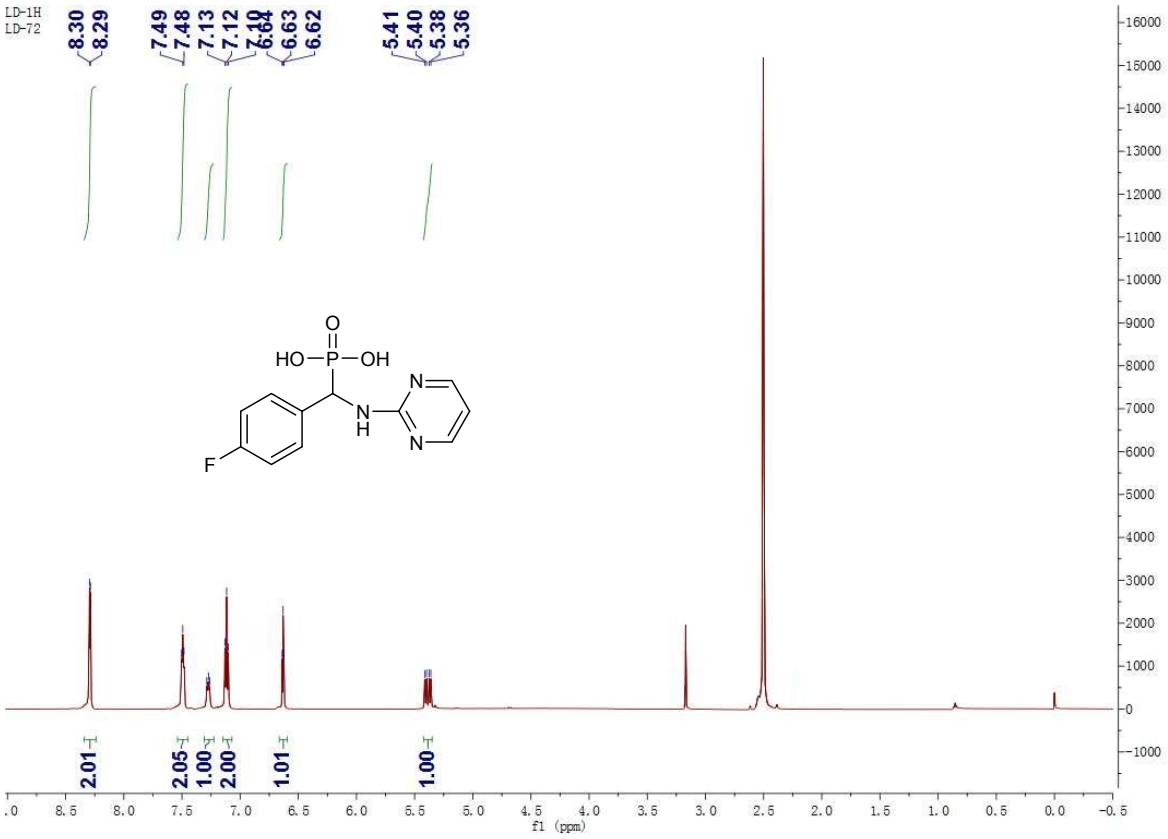


HRMS Spectrum

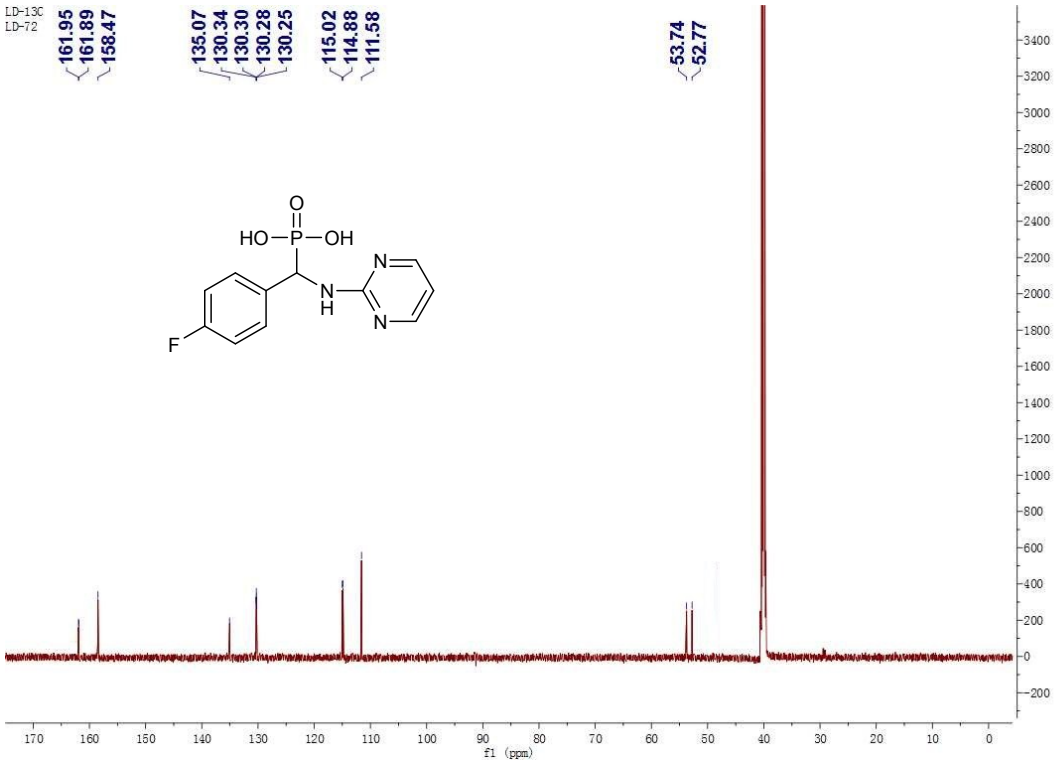


6.10 Spectra of compound 5b

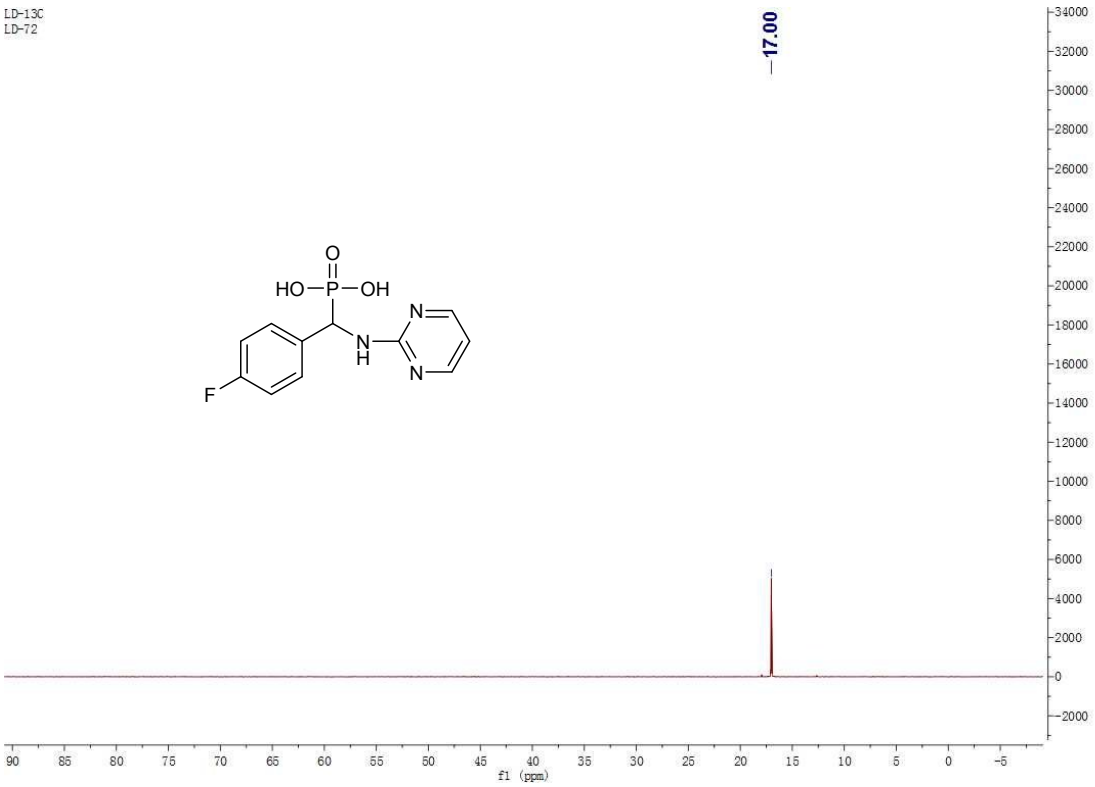
^{1}H NMR Spectrum



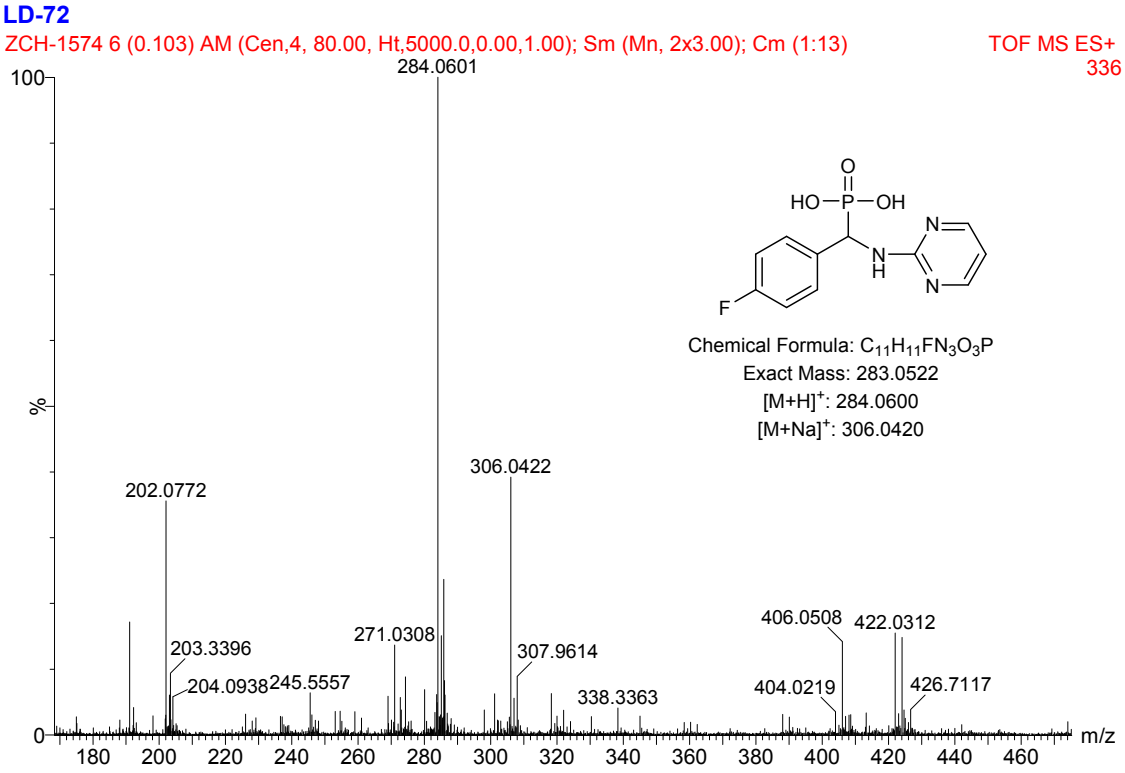
^{13}C NMR Spectrum



^{31}P NMR Spectrum

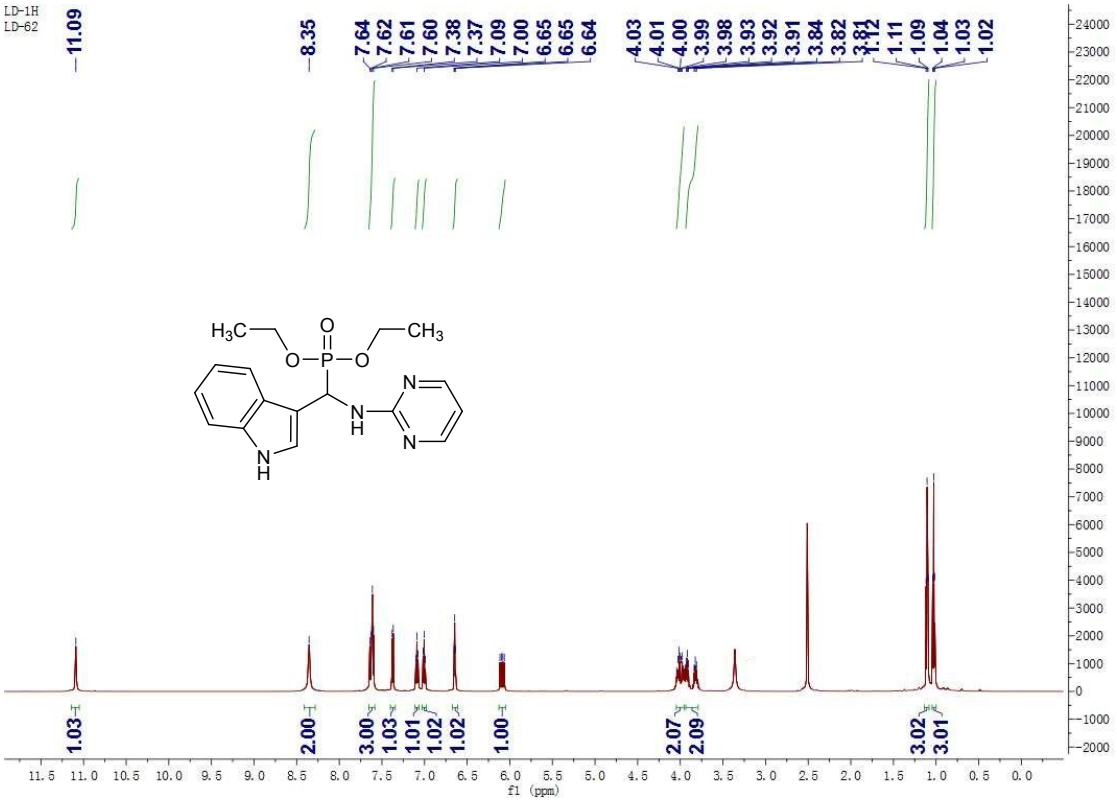


HRMS Spectrum

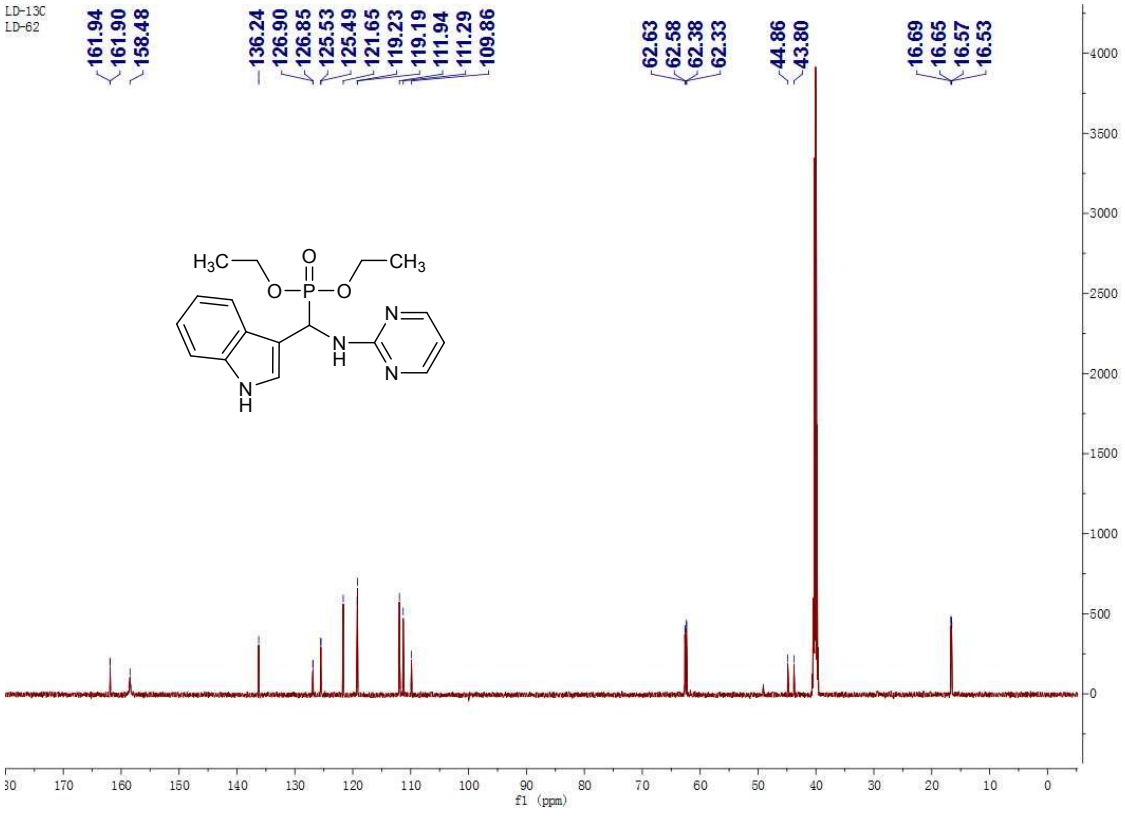


6.11 Spectra of compound 6a

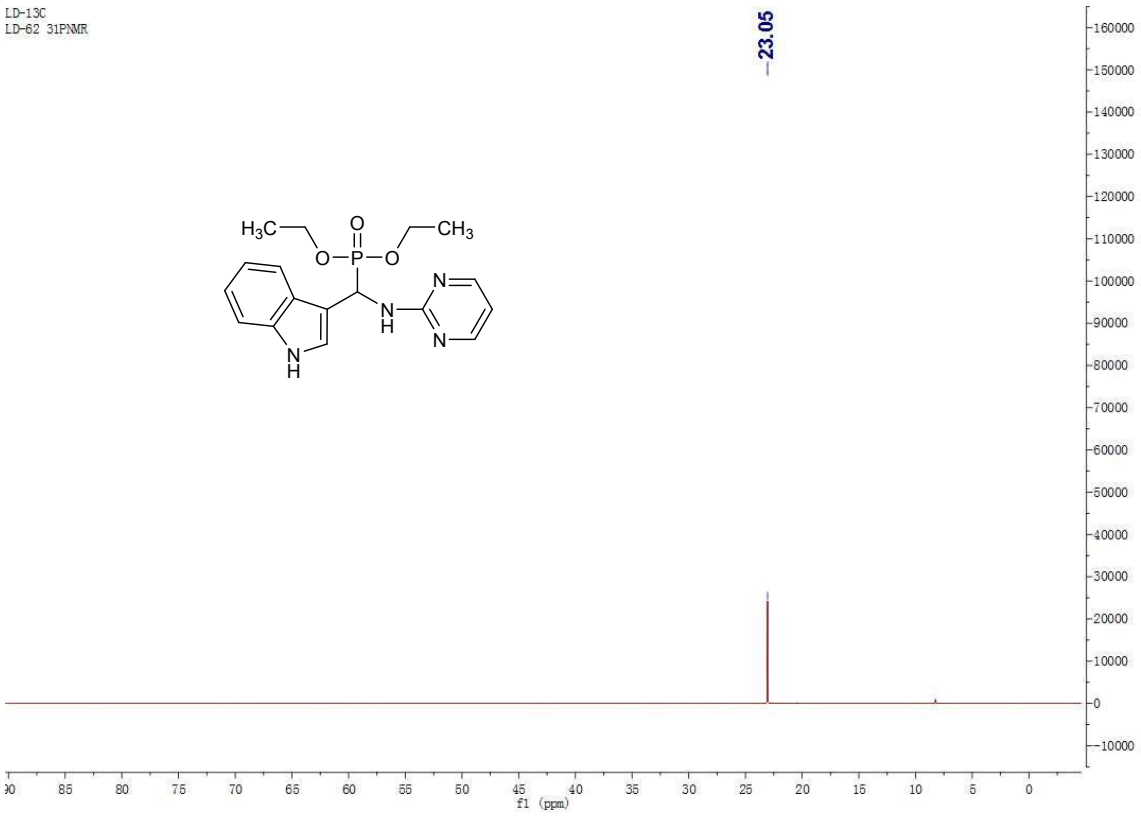
^{1}H NMR Spectrum



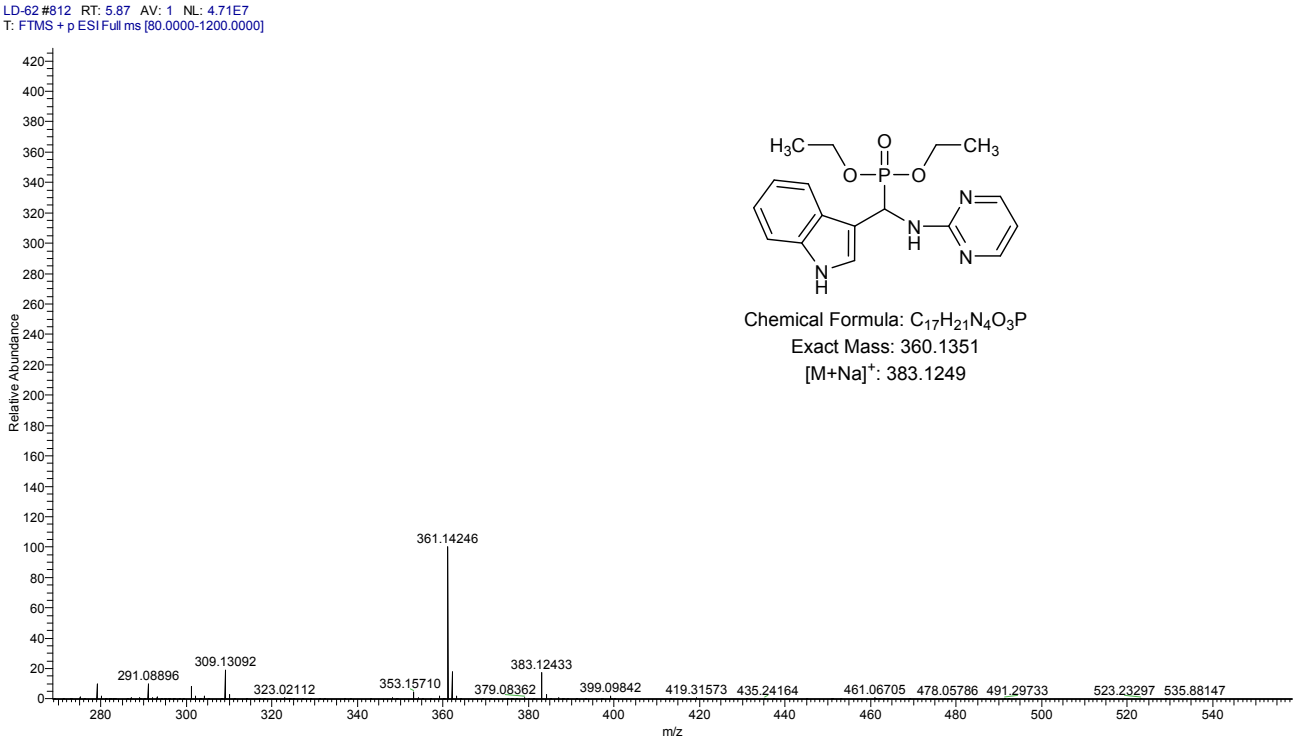
^{13}C NMR Spectrum



^{31}P NMR Spectrum



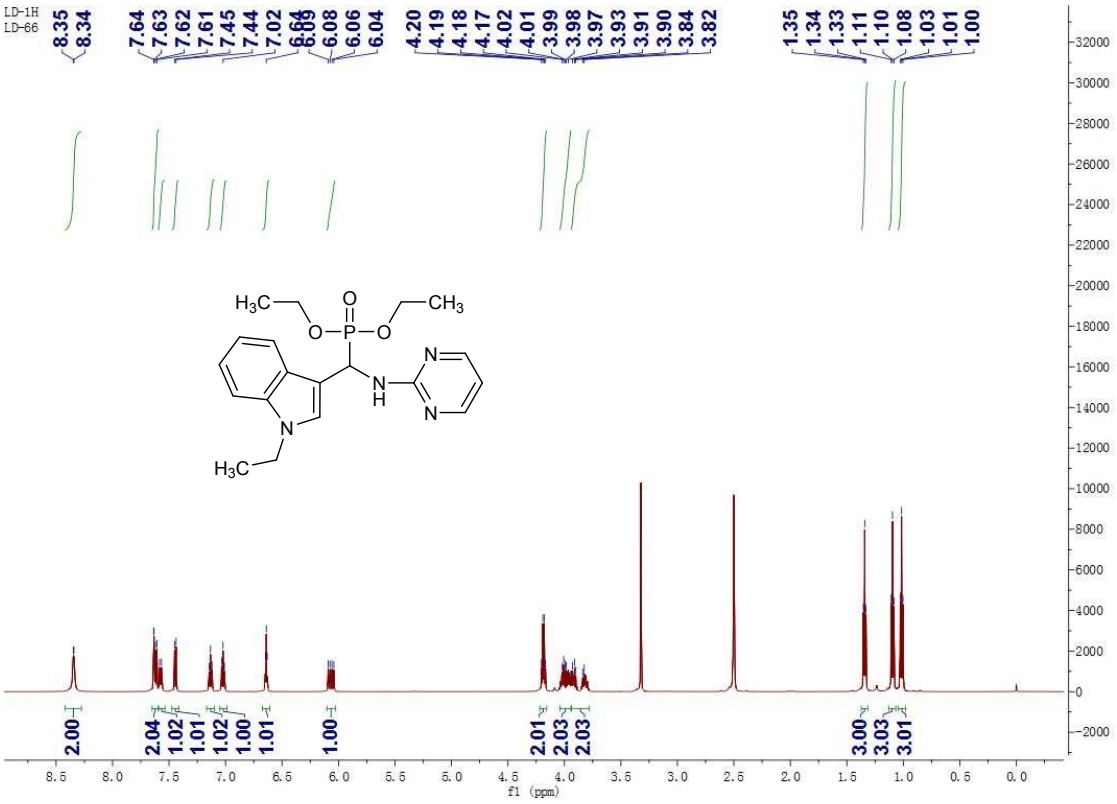
HRMS Spectrum



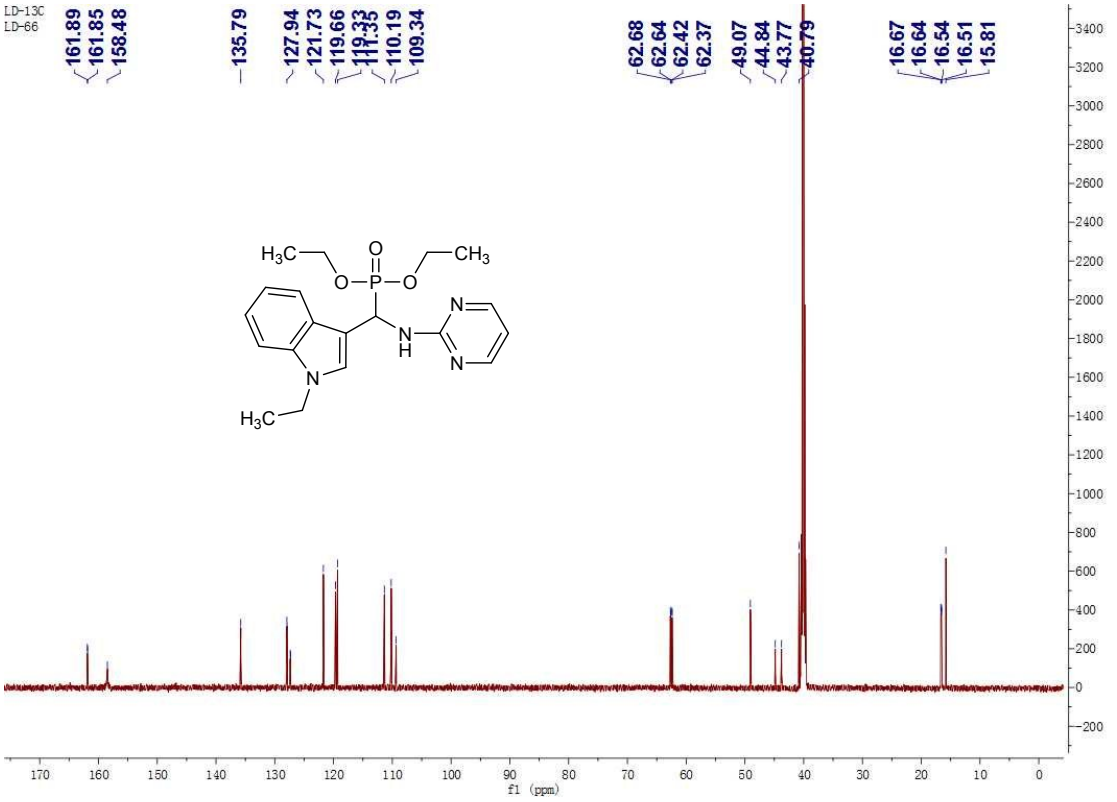
Chemical Formula: $C_{17}H_{21}N_4O_3P$
Exact Mass: 360.1351
$[M+Na]^+$: 383.1249

6.12 Spectra of compound 6b

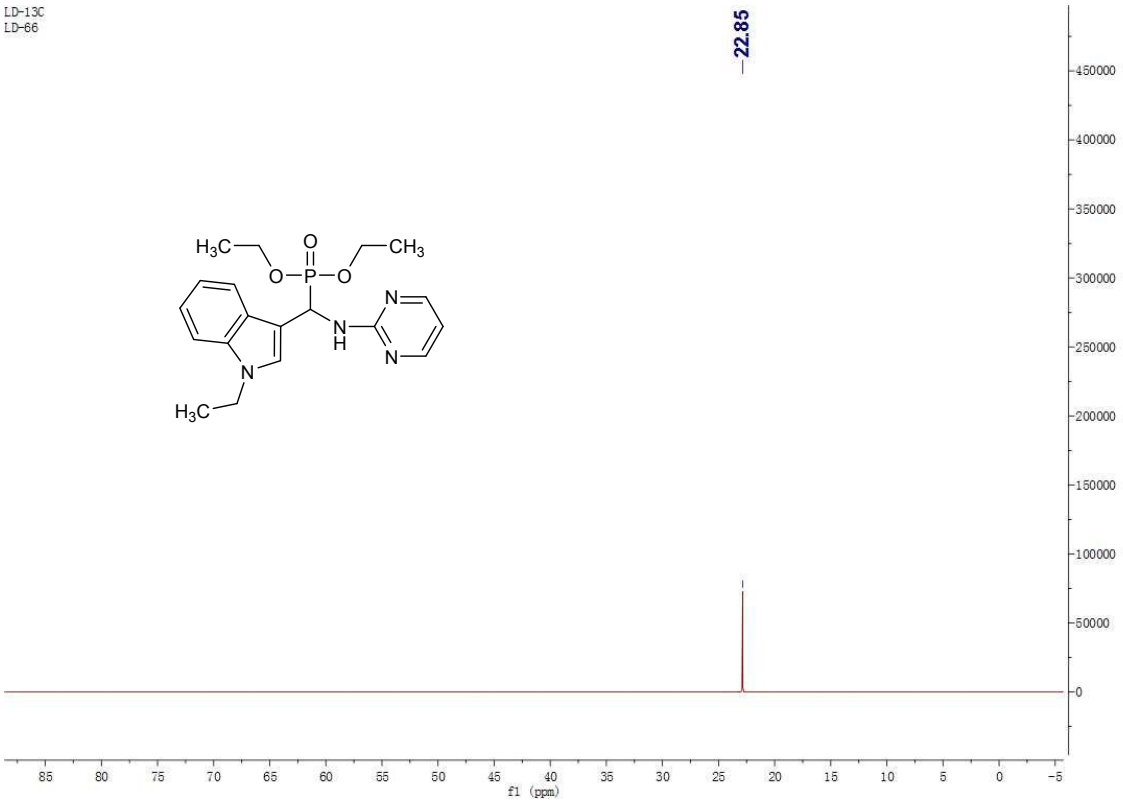
^{1}H NMR Spectrum



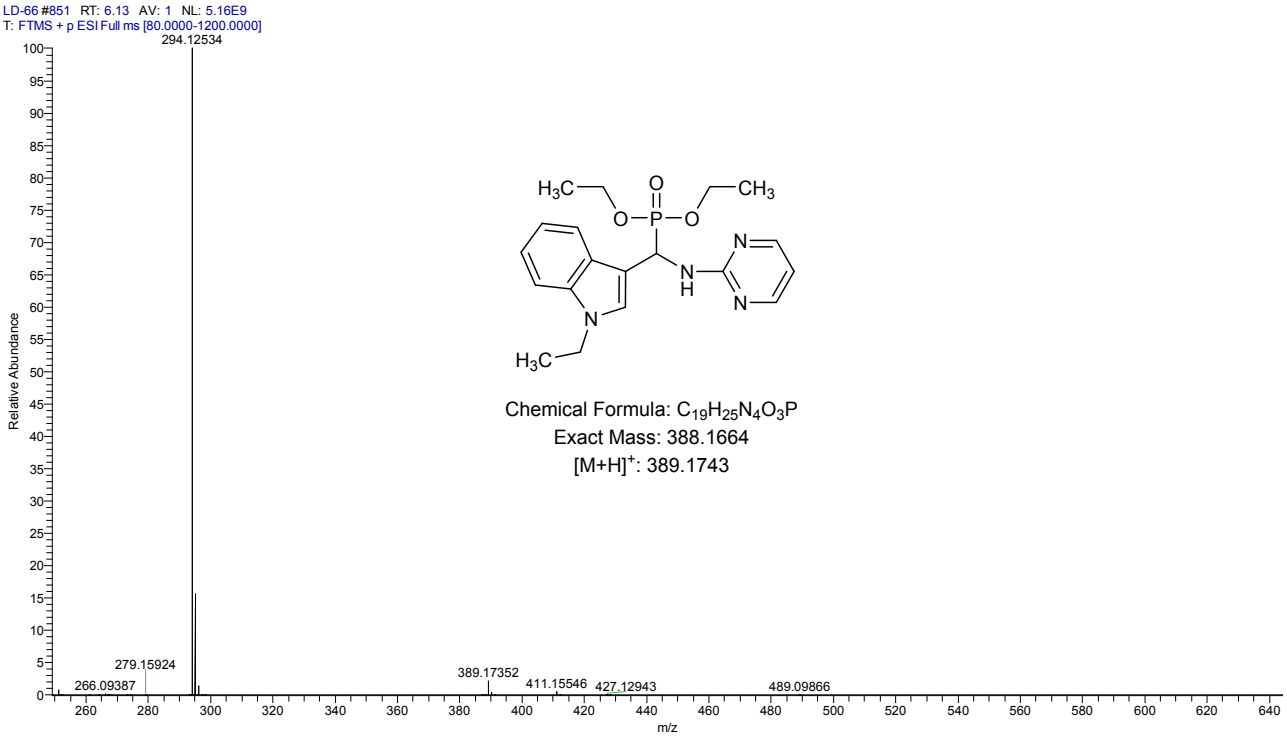
^{13}C NMR Spectrum



^{31}P NMR Spectrum



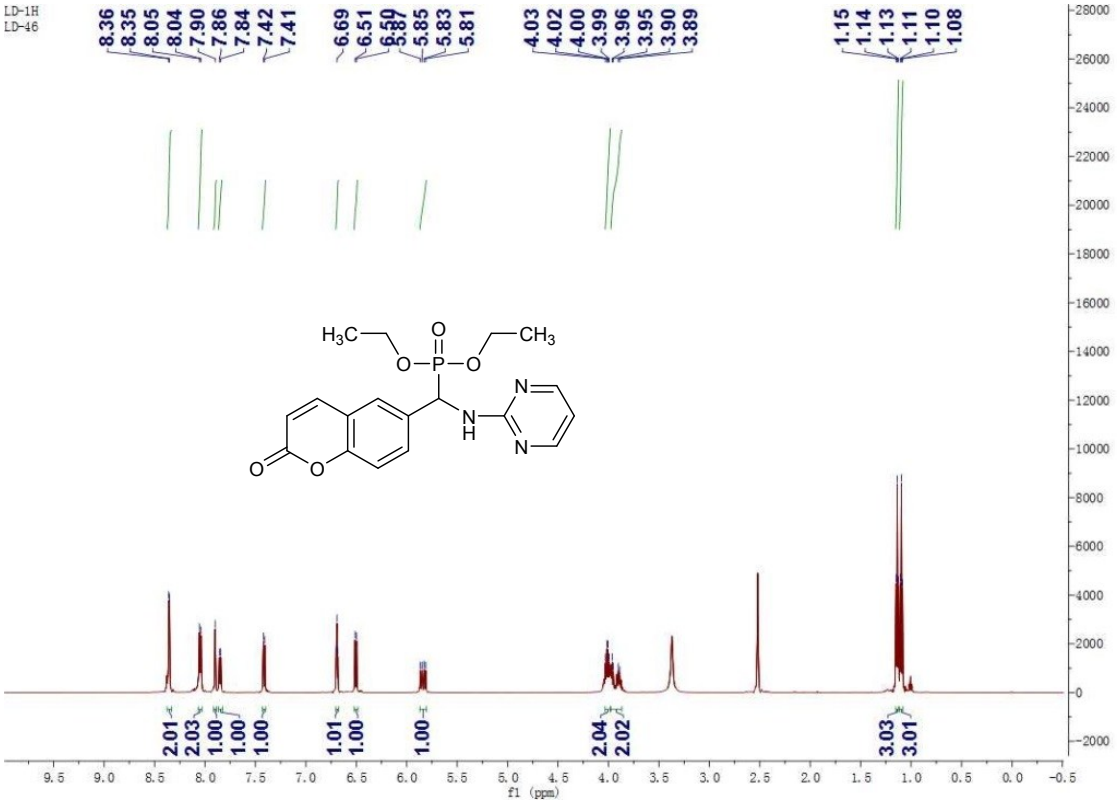
HRMS Spectrum



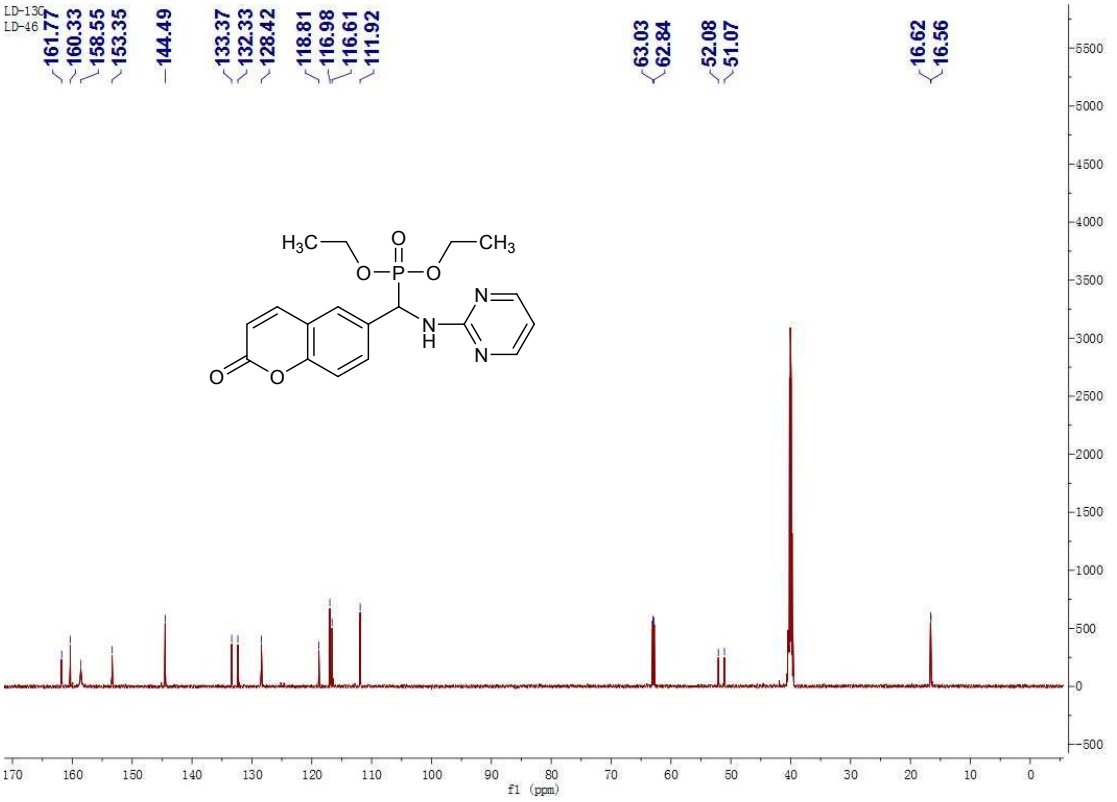
Chemical Formula: $C_{19}H_{25}N_4O_3P$
Exact Mass: 388.1664
$[M+H]^+$: 389.1743

6.13 Spectra of compound 7

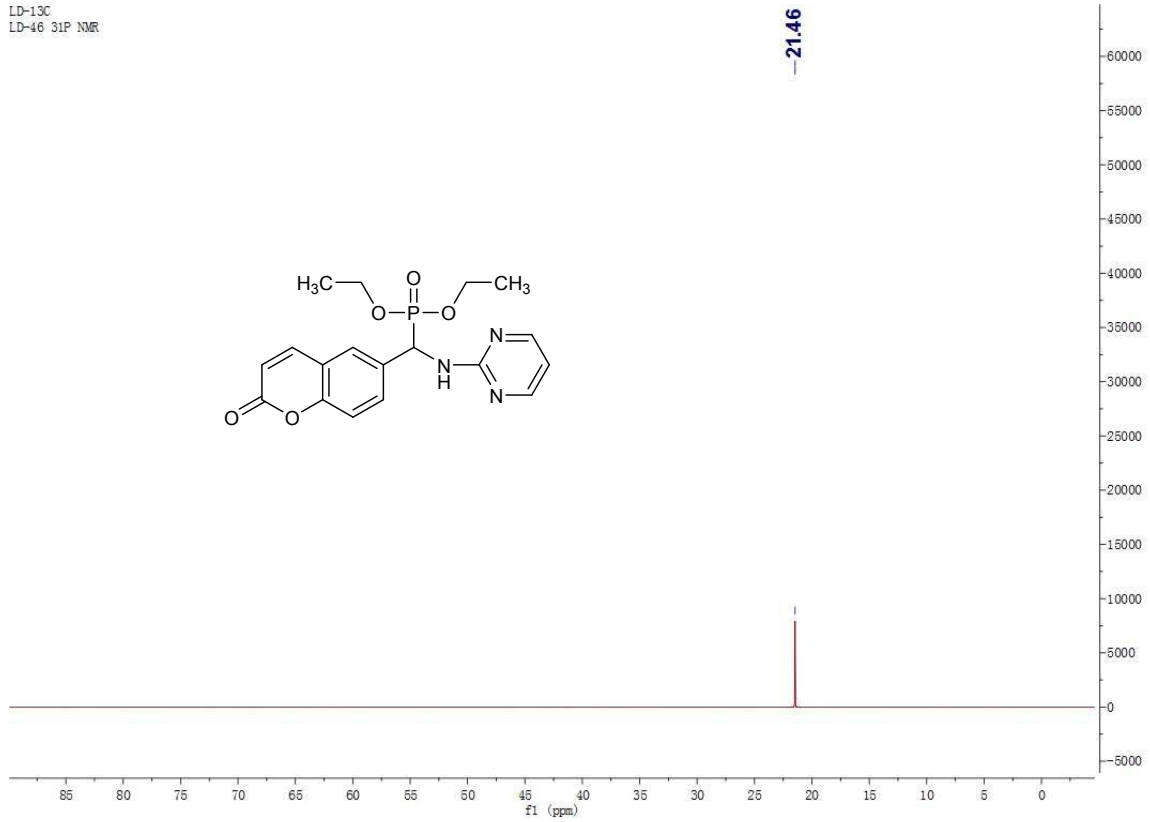
^{1}H NMR Spectrum



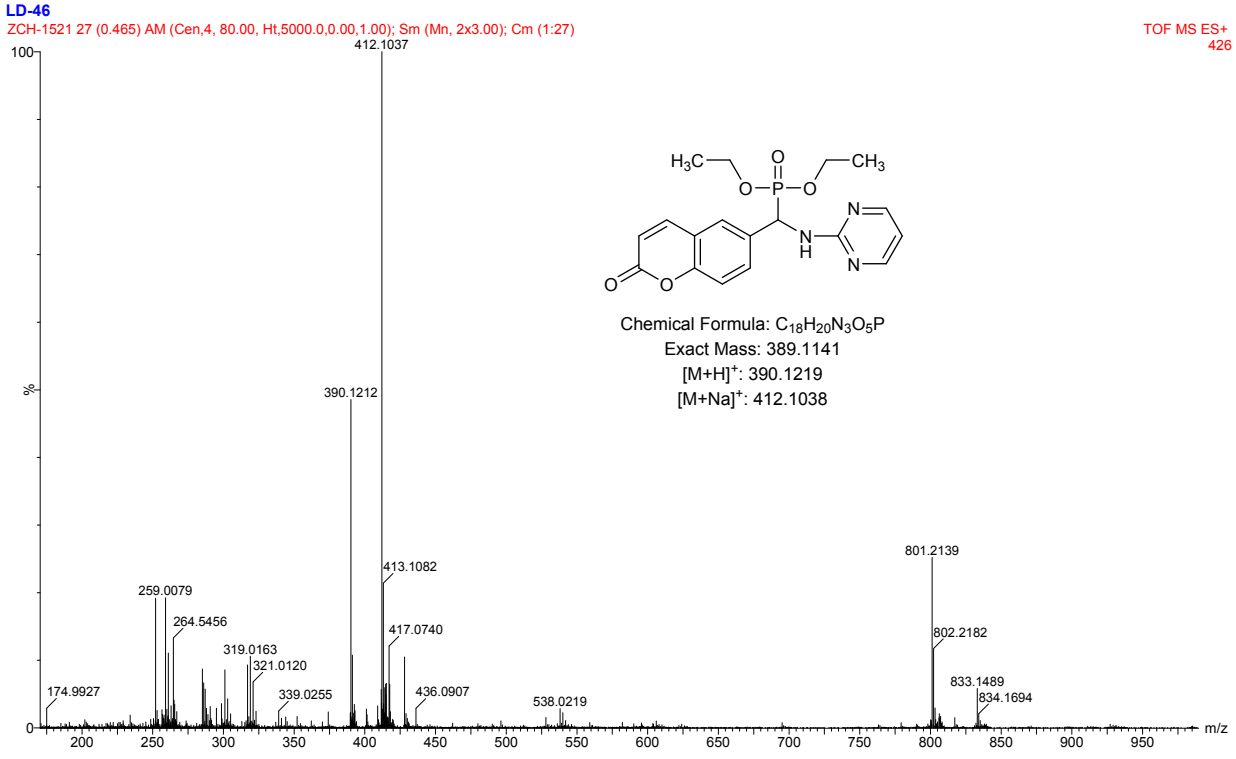
^{13}C NMR Spectrum



^{31}P NMR Spectrum

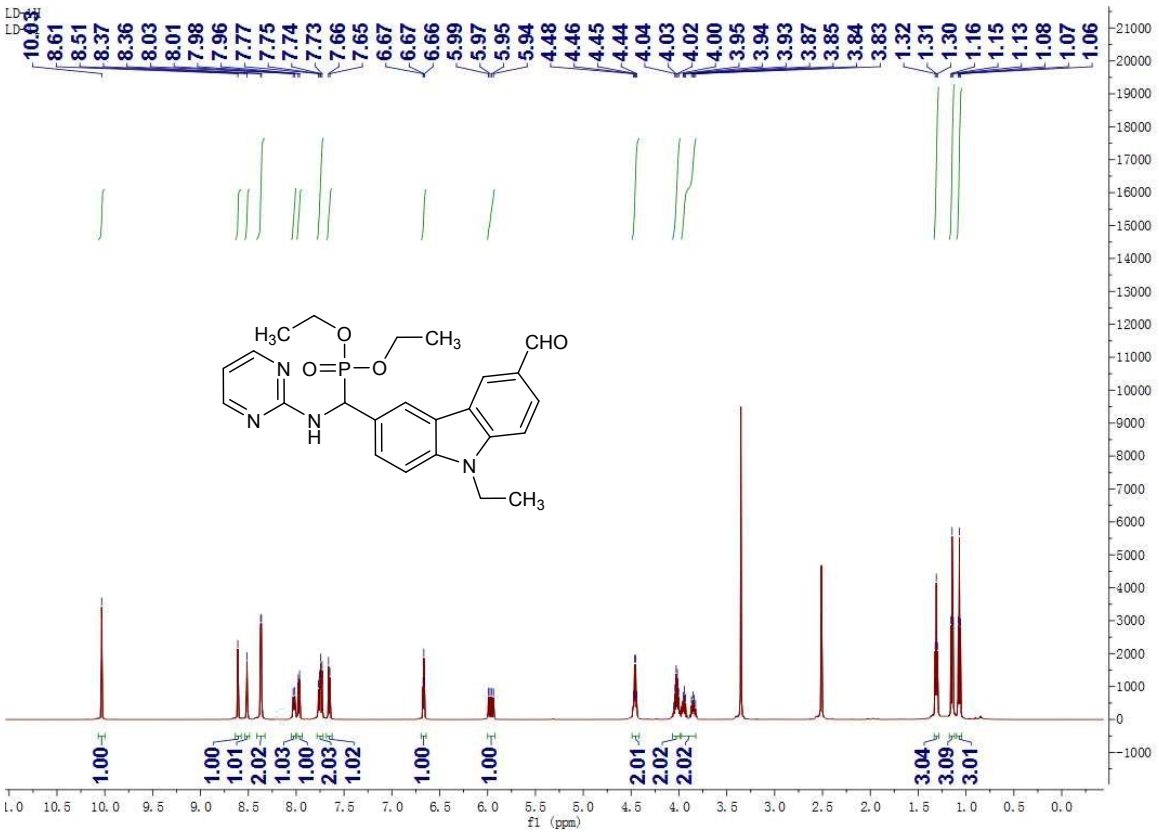


HRMS Spectrum

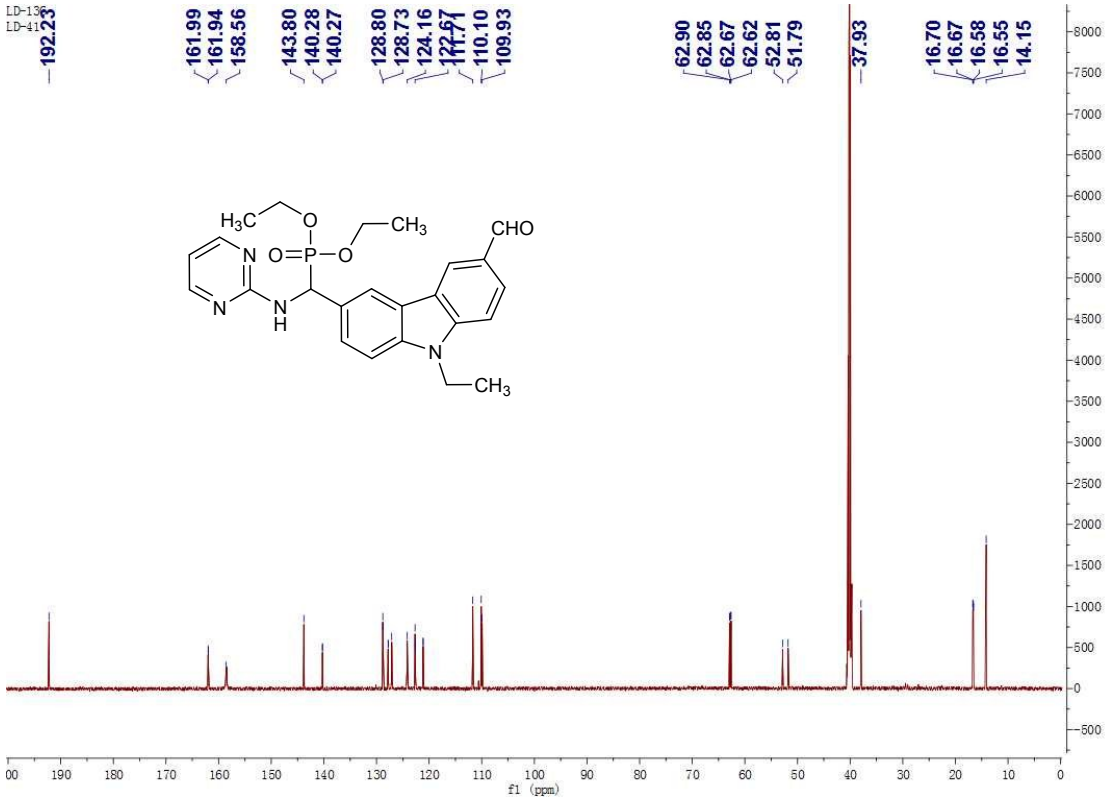


6.14 Spectra of compound 8

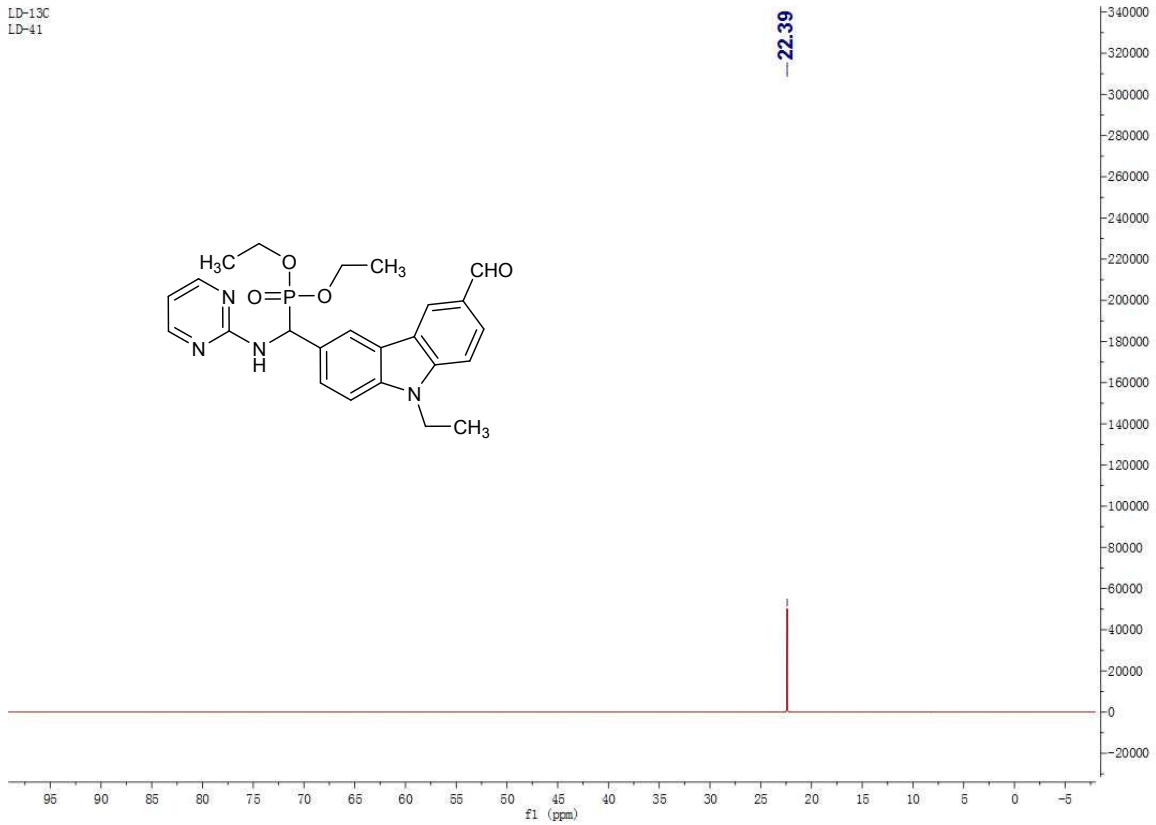
^{1}H NMR Spectrum



^{13}C NMR Spectrum



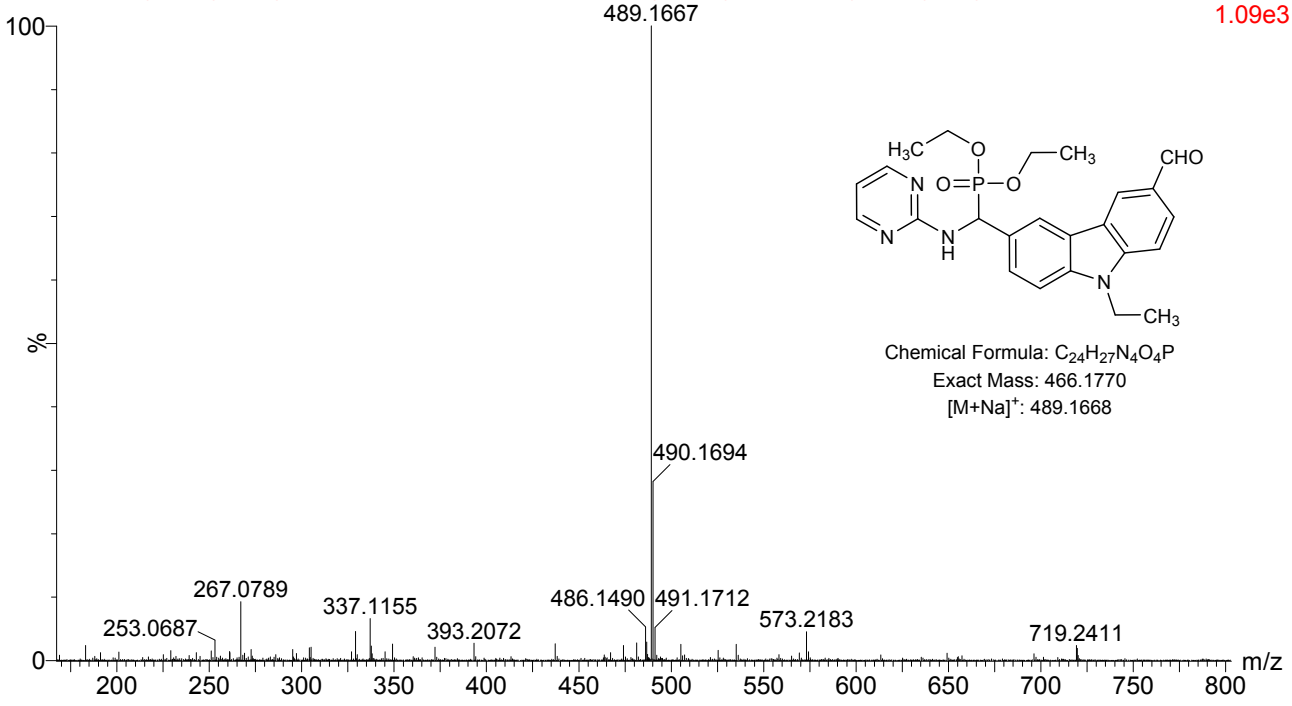
^{31}P NMR Spectrum



HRMS Spectrum

LD-41

ZCH-1448 4 (0.069) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:16) TOF MS ES+ 1.09e3



Chemical Formula: $C_{24}H_{27}N_4O_4P$
Exact Mass: 466.1770
$[M+Na]^+$: 489.1668